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And

GNS Down Syndrome Foundation
Autism Care Network Foundation
Newborn Foundation of Azerbaijan
GNS Bangladesh Newborn Foundation
GNS Foundation of Germany
Global Newborn Society Foundation of Italy
Mongolian Association of Obstetrics Gynecology and Neonatology
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The organization, Protecting Brains and Saving Futures, Brasil
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Polish Nursing Association - Płock, Poland
Panlibyan Neonatal Association
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GNS Association for Perinatal Care
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Sociedad Latinoamericana de Residentes de Neonatologia (SolaReNeo)
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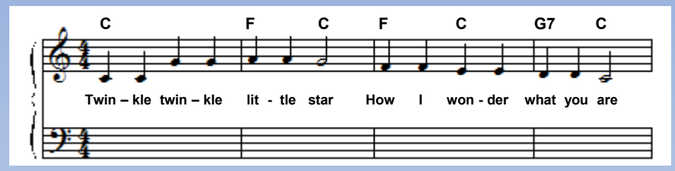
Anatolian Midwives Association
The Organization, First Breaths of Life
GNS Western Australia
Perinatal Society of Singapore
Pioneers
Bhutan Neonatal Care Forum

Highlighted articles:



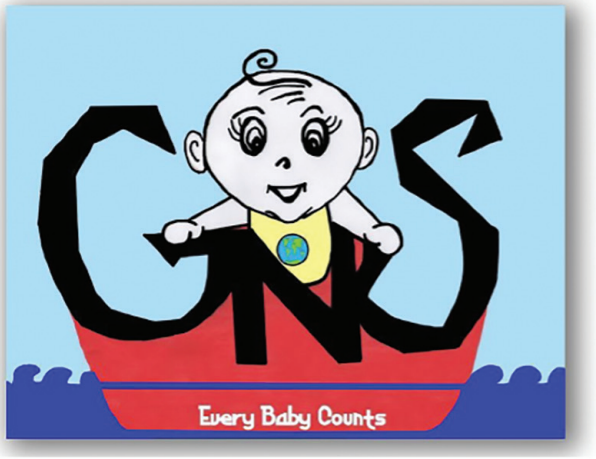
Fetuses can Listen, Learn, and Remember: We Need to be Cautious about What and How We Say It!

Epigenetics of Down Syndrome

Navigating Information Overload on Social Media: Opportunities and Misadventures for Clinicians and Professionals

Also available online at
https://www.globalnewbornsociety.org/our-scientific-journal-newborn

Bibliographic Listing:
J-Gate, Scilit, WorldCat,
PORTICO, ICMJE



Global Newborn Society

Each time we lose an infant, we lose an entire life and its potential!

Newborn is the official journal of the Global Newborn Society (GNS), a globally active, non-profit organization that is registered as a 501(c)(3) non-profit formation in the United States and is currently being listed as an analogous charity in many other nations. The aim is to enhance research in newborn medicine, understand epidemiology (risk factors) of disease, train healthcare workers, and promote social engagement. The GNS was needed because despite all improvements in medical care, infants remain a high-risk patient population with mortality rates similar to 60-year-olds. We need to remind ourselves that *Every Baby Counts*, and that *Each Time We Lose an Infant, We Lose an Entire Life and its Potential*.

Our logo above, a hand-drawn painting, graphically summarizes our thought-process. There is a lovable little young infant exuding innocent, genuine happiness. The curly hair, shape of the eyes, long eyelashes, and the absence of skin color emphasize that infants need care all over the world, irrespective of ethnicity, race, and gender. On the bib, the yellow background reflects happiness, hope, and spontaneity; the globe symbolizes well-coordinated, worldwide efforts. The age-related vulnerability of an infant, with all the limitations in verbal expression, is seen in being alone in the boat.

The unexpressed loneliness that many infants endure is seen in the rough waters and the surrounding large, featureless sky. However, the shades of blue indicate that the hope of peace and tranquility is not completely lost yet. The acronym letters, GNS, on the starboard are made of cast metal and are pillars of strength. However, the angular rough edges need continued polishing to ascertain adequacy and progress. The red color of the boat symbolizes our affection. The expression "*Every Baby Counts*" seen on the boat's draft below the waterline indicates our commitment to philanthropy, and if needed, to altruism that does not always need to be visible. The shadow behind the picture shows that it has been glued on a solid wall, one built out of our adoption and commitment.

Design of the Journal Cover

The blue color on the journal cover was a careful choice. Blue is the color of flowing water, and symbolizes the abnormalities of blood vascular flow that are seen in many neonatal illnesses. There is a gradual transition in the shades of blue from the top of the cover downwards. The deeper shades of blue on the top emphasize the depth, expertise, and stability, which the renowned authors bring. Light blue is associated with health, healing, tranquility, understanding, and softness, which their studies bring. The small letter "*n*" in the title of the journal, *newborn*, was chosen to emphasize the little size of a newborn baby. The issue editors chose three articles to be specifically highlighted; the two pictures and two titles below reflects an order suggested by them.

Instructions to Authors

The journal welcomes original articles and review articles. We also welcome consensus statements, guidelines, trials methodology, and core outcomes relevant to fetuses/young infants in the first 1000 days. A detailed set of instructions to authors can be seen online at https://www.globalnewbornsociety.org/intructions-for-authors. The manuscripts can be submitted via the online manuscript submission system.

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Contents

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EDITORIAL

We Need to Come Together to Save Premature Infants

Premature infants are at a high risk of morbidity and mortality.[1] Around 13.4 million babies are born before 37 weeks of pregnancy each year,[2] which add up to about 1 in 10 infants born worldwide.[3] Of these, we lose about 1 million each year.[4] To remind ourselves about this risk each year, we observe November 17th as the World Prematurity Day.[5] Many organizations, including the European Foundation for the Care of Newborn Infants and allied groups;[6] LittleBigSouls (Africa);[7] March of Dimes (USA);[8] and the Miracle Babies Foundation[9] and Running for Premature Babies Foundation[10] (both from Australia) have drawn the world's attention to infants' needs for many years.

The Global Newborn Society (GNS), its partnering Mozib Center for Neonatal Care, and 37 allied organizations with members from 133 countries have now joined this movement: the GNS Down Syndrome Foundation, Autism Care Network Foundation, Newborn Foundation of Azerbaijan, GNS Bangladesh Newborn Foundation, GNS Foundation of Germany, Global Newborn Society Foundation of Italy, Mongolian Association of Obstetrics, Gynecology and Neonatology, Foundation for Human Milk Feeding in the Islamic World, The organization - Protecting Brains and Saving Futures in Brasil, Association of Neonatologists in the United Kingdom, Polish Nursing Association – Płock, Panlibyan Neonatal Association, Association for Indigenous Peoples in India, Association for Newborn Care in Pakistan, GNS Association for Perinatal Care, Association for Infant Nutrition in the Middle East, Sociedad Latinoamericana de Residentes de Neonatologia (SolaReNeo), Uruguayan Neonatal Association, Paraguayan Society of Pediatrics Committee for Neonatology, Armenian Association of Neonatal Medicine, Association of Pediatricians of Uzbekistan, GNS Cardiology Association of Iraq, Iranian Forum for Infant Nutrition, Nepalese Association for Newborn Health, GNS Forum for Transgenerational Inheritance, PreemieWorld Foundation, GNS Forum for Data Analytics, GNS Forum for Nanomaterials, Neonatology Branch of the Chilean Pediatric Society, Carlo GNS Center for Saving Lives at Birth, Dudeja GNS Center for Infectious Diarrheal Diseases, Anatolian Midwives Association, The Organization - First Breaths of Life, GNS Western Australia, Perinatal Society of Singapore, Pioneers, and the Bhutan Neonatal Care Forum. The shared goal is to minimize infant morbidity and mortality throughout the world. At the end of the day, *Every Baby Counts*![11]

In this same 4th quarter of the year, December 3 is recognized as the International Day of Persons with Disabilities.[5] Here, premature infants are again at a high risk of disabilities;[12-14] about a third born at or prior to 24 weeks of pregnancy may show neurodevelopmental delay,[12,15] cerebral palsy,[16] slow cognitive development,[17] vision and hearing impairment,[18] and/or learning disorders.[19] These infants may also show poor growth[20] and limitations in communication[21] and in social development.[22,23] The risk of disability increases with the degree of prematurity and the severity of peri-/postnatal illness.[24] And babies—be it the Global North or South—do not talk,[25] or vote,[26] and hence, need someone to help.[4, 27-32]

We would like to make a special announcement here. The first conference of the GNS has been tentatively scheduled for November 3-5, 2025 in Stockholm, Sweden (https://www.globalnewbornsociety.org/our-first-conference). We thank our leaders for their efforts; many have committed their personal funds for the conference.

The leadership of the GNS has decided to develop several subcommittees with representation from each of the six populated continents. These groups will be focused on: (a) leadership and policy of the organization; (b) infant nutrition; (c) problems unique to tropical/peri-equatorial countries; (d) social engagement with families of critically ill infants; and (e) standardization of tools such as functional ultrasound. Each subcommittee will organize important sessions in our upcoming conference.

The editorial team has decided to highlight one of our partnering members in each issue of the journal. For the first, we are particularly proud to have been chosen by the Bhutan Neonatal Care Forum as their official mouthpiece. Bhutan is an ancient culture located in the eastern Himalayas in South Asia that is renowned for measuring its progress using Gross National Happiness,[33] not the usual financial indices. The terrain is particularly difficult with the highest mean ruggedness values in the world at approximately 768 meters and an average altitude of 3,280 meters (10,761 feet) above sea level.[34] As of 2023, the total recorded population in this region was 7,77,224, averaging at 21 people per square kilometer. The projected birth rate of 16.118 births per 1,000 people is lower than the 19.016 deaths per 1,000 live births, indicating a negative growth rate of the population (data from the Bhutan government). The difficulties in transport of critically ill infants, personnel, equipment, and drugs in the mountainous terrain can only be imagined. Now, with strong commitments from the public and its government, the country is taking important steps to develop newborn care in the region. The two qualified neonatologists in the country are located primarily in the Central Newborn Facility in Thimphu and are actively developing a workforce to treat sick infants. **Figures 1A and B** show two images from the recent celebrations of the World Prematurity Day in Bhutan. There will be more details in the following issues.

Our journal, the *newborn*, aims to cover fetal/neonatal problems that begin during pregnancy, at the time of birth, or during the first 1,000 days after birth.[35] As in our previous issues, we again present 8 articles here **(Fig. 2)**. The first is another one in our Down syndrome (DS) lineage of studies.[36] The high levels of clinical heterogeneity seen in DS are surprising when we consider the fairly restricted genetic alteration in these infants;[37-39] nearly 95% patients have a simple triplication of chromosome 21 (*Homo sapiens* 21, Hsa21) and the remaining 5% may carry translocations or mosaicism of its segments.[40-42] In this article, the authors examined existing information on epigenetic modifications—this is an attractive area for study because many epigenetic marks may be reversible with opportunities for therapeutic interventions.[43,44] These epigenetic changes could be genome-wide and include DNA methylation, post-translational histone modifications, and histone core variants.[45,46] Existing data emphasize two trans-acting molecular mechanisms: (a) tissue-specific histone modifications that can alter the expression of regulatory genes;[47] and (b) the effect of genes that are expressed in Hsa21q21.[48-50] This article provides useful information for understanding the phenotypic variability in DS.

Figs 1A and B: Celebrations of World Prematurity Day in Bhutan in 2024.

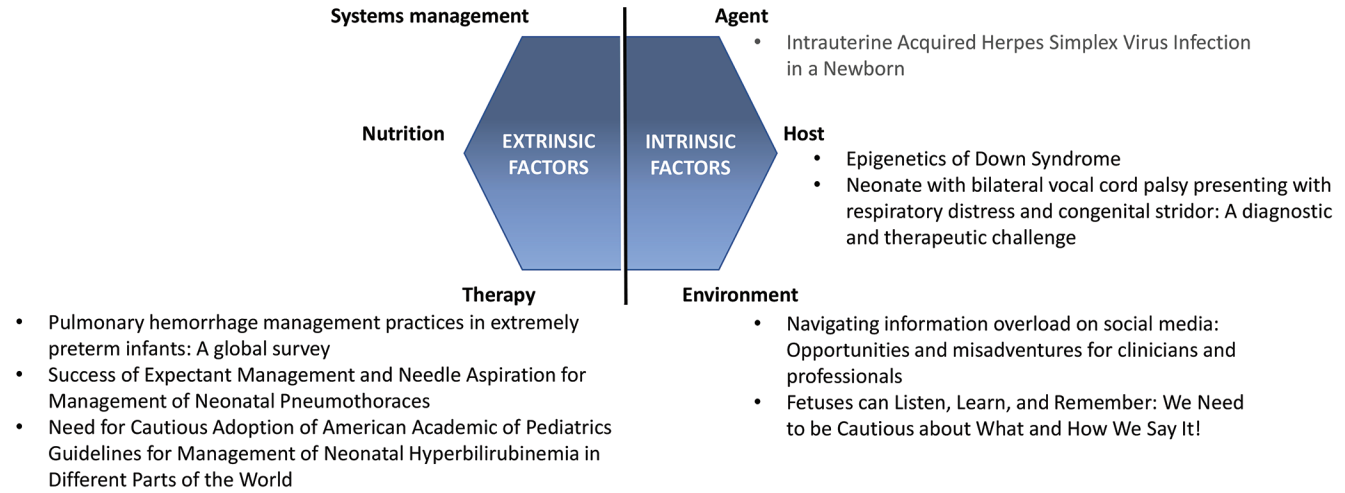

Fig. 2: Areas of focus in the *newborn*, Volume 3, Issue 4. We have expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three additional foci represent extrinsic factors that can affect health—those originating in therapy, nutrition, and systems management are shown. This issue covers 4 nodes, those related to an agent, host factors, environment, and treatment/monitoring systems.

Thakkar et al.[51] performed a global survey on the management of pulmonary hemorrhage (PHEM), which can be a life-threatening condition in extremely premature infants. We do not have any specific treatment for this condition.[52] A multi-institutional working group of physicians designed a 14-question survey around their experience and current controversies reported in the literature. There were 360 responses from 73 countries. Most neonatologists managed PHEM without unit-based guidelines. There was a consensus on using endotracheal epinephrine, blood products and high-frequency oscillatory ventilation. More participants administered surfactant after rather than during acute PHEM. Later, most obtain echocardiograms and consider treatment for PDA with acetaminophen. There is need for high-quality, evidence-based guidelines to provide appropriate care to these patients.

There is evidence that the fetal auditory system begins to show functional activity during mid-gestation or possibly even earlier.[53,54] Frydrysiak-Brzozowska and coworkers[55] examined existing information that fetuses can respond to maternal voice and different types of music, both vocal and instrumental. Interestingly, fetuses may not only recognize but also retain some memory of these auditory stimuli. There is a need for well-designed randomized-controlled trials to compare different durations and types of musical (sound) intervention. These interventions can show neurodevelopmental gains; at a minimum, there could be improvement in maternal-fetal bonding.[56] There might also be some benefit in conditions such as neonatal abstinence syndrome.[57] Carefully conducted studies of intrauterine neurosensory organization with long-term follow-up can be helpful.[58]

Shahrour et al.[59] examined that efficacy of early hospital discharge (<72 hours following birth) of healthy term and near-term infants. It is favored to promote family care but may have increased hyperbilirubinemia-related hospital readmissions.[60] The authors performed a retrospective single-center study to compare the 2009 and 2022 American Academy of Pediatrics (AAP) discharge guidelines[61,62] in the United Arab Emirates (UAE) for the impact on hyperbilirubinemia-related readmissions. They reviewed records from 895 infants born with a gestational age (GA) ≥ 35 weeks and a birth weight (BW) ≥ 2500 grams and GA ≥ 36 weeks/BW ≥ 2000 grams. Infants were classified into risk zones based on predischarge transcutaneous bilirubin (TcB) or total serum bilirubin (TSB) levels (AAP 2009 hyperbilirubinemia nomograms). They noted that unlike in the United States, the 2022 guidelines would have recommended more infants for follow-up visits within 2 days than the 2009 recommendations; the need for phototherapy would also have been higher. However, the frequency of severe hyperbilirubinemia requiring phototherapy would have remained similar. They recommend that the AAP early hospital discharge guidelines need to be specifically evaluated in different ethnic groups in various parts of the world.

Spillane and colleagues[63] examined the efficacy and safety of expectant management and needle aspiration as definitive, not just as temporizing, treatment options for neonatal pneumothorax (PTX).[64] This was a retrospective single center study of 114 PTX; 20.2% were treated with chest tube drainage, 25.4% with needle aspiration, and 54.4% with expectant management. The efficacy of expectant observation was 96.8%, needle aspiration was 37.9%, and chest tube drainage was 91.3. Needle aspiration and chest tube drainage were more frequently utilized for moderate, late, or tension PTX. The authors concluded that most small PTXs resolve spontaneously over time and just need expectant observation. Needle aspiration was utilized in less mature neonates with more complex PTXs; it was safe and helped avoid more invasive chest tube placement in a significant number of neonates.

Vereen et al.[65] reviewed the impact of correct vs. misinformation (false or inaccurate information) or disinformation (deliberate attempt to mislead) from social media on families.[66,67] Consequently, there are ever-increasing difficulties in practicing evidence-based medicine. This article describes how health professionals can recognize appropriate information on social media and stay updated with consequent risks/benefits. Certain best use practices have been appraised critically. Development of information-sharing paradigms can help in effective collaboration with colleagues and communication with families.

Ocampo-Chih and colleagues[68] have presented a fatal case of herpes simplex virus (HSV) infection in a premature infant following *in utero* exposure in a nonimmune mother.[69] Most vertical maternal-to-fetal HSV transmissions occur during or after birth.[70] In this report, the authors have described a 30-week pregnant woman who had a history of flu-like illness at 18 weeks, and then reported a reduction in fetal movements and vaginal bleeding; fetal ultrasound evaluations showed an echogenic bowel, polyhydramnios, and then brain abnormalities. At birth, the infant had extensive skin desquamation, seizures, and brain malformations. HSV-2 was detected in the infant's blood. This case is important because maternal treatment with antiviral medications and cesarean section can prevent perinatal but not *in utero* infections. As no HSV vaccines are available yet,[71] protecting nonimmune pregnant women from HSV exposure is the only way to prevent vertical transmission.

In another article, Patel et al.[72] have described a full-term infant with biphasic stridor.[73] He had an uneventful pre- and perinatal course but developed respiratory distress immediately after birth and needed assistive ventilation. There was no remarkable lung disease. The clinicians were able to wean him to non-invasive respiratory support within 48 hours, but noted persistent biphasic stridor with high work-of-breathing. Flexible and rigid bronchoscopy showed bilateral vocal cord palsy (VCP).[74] The vocal cord movement did not change over time and a tracheostomy was performed on postnatal day 38. He was successfully discharged home a few days later. The vocal cord movement did not improve over the next several months. A comprehensive evaluation showed his laryngeal dysfunction to be an isolated, primary clinical problem. The authors have discussed the differences in the clinical course, need for surgery, and outcomes in infants with primary vs. syndromic VCP.

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ORIGINAL RESEARCH

Success of Expectant Observation and Needle Aspiration in Reducing the Need for Chest Tube Drainage for Management of Neonatal Pneumothoraces

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Abstract

Aim: Pneumothorax (PTX) is a common morbidity during the newborn period. The aim of this study is to determine the efficacy and safety of expectant observation (EO) and needle aspiration as definitive treatment options for neonatal pneumothoraces.

Materials and methods: This is a retrospective single-center study from 2017 to 2019 of 114 PTX. Maternal, neonatal, and PTX characteristics were examined and associations between type of intervention, efficacy of intervention and patient/PTX characteristics were assessed.

Results: For primary treatment, 20.2% of PTX were treated with chest tube drainage (CTD), 25.4% with needle aspiration (NA), and 54.4% with EO. The efficacy of primary treatment was 91.3% with CTD, 37.9% with NA, and 96.8% with EO. NA and CTD were utilized more frequently than EO for moderate PTX (59.3 vs 40.9 vs 13.1%, $p < 0.001$), late PTX (75.9 vs 78.3 vs 27.4%, $p < 0.001$), and PTX with tension (41.4 vs 39.1 vs 1.6%, $p < 0.001$). In multivariate analysis, NA was the only factor associated with significantly lower success [adjusted odds ratio (OR) 0.08, 95% confidence interval (CI) 0.02–0.40]. None of the infants experienced any complications.

Conclusion: Expectant observation was the most frequent treatment modality and highly successful in management of small PTX. NA was utilized in less mature neonates with more complex PTX; it was safe and avoided more invasive CTD in a significant percentage of neonates.

Clinical significance: In our experience, EO and NA were highly safe, efficacious, and definitive management strategies of small pneumothoraces, not just a temporizing procedure to stabilize these infants prior to eventual CTD. CTD, which is much more invasive, was required only in a small fraction of these patients and further study is needed to define its indications.

Keywords: Chest tube drainage, Expectant observation, Needle aspiration, Neonates, Pneumothorax, Premature neonates.

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Introduction

Pneumothorax (PTX; pulmonary air leak) is seen more frequently in neonates than in any other age-group; the incidence can be as high as 4–6% in preterm and 0.2–2% in all neonates.[1,2] These patients are at a higher risk of PTX because of multiple factors, including high transpulmonary pressures generated with the first breaths, rapidly changing pulmonary compliance, frequent need for positive pressure in the delivery room (DR), and high incidence of respiratory disease.[3] Of concern, the recent rise in the use of positive pressure in the DR has been associated with increased neonatal PTX.[4] Timely identification and management of neonatal PTX is critical; it can be associated with respiratory failure and cardiovascular collapse, need for prolonged respiratory support and hospitalization, and death.[3] In addition, preterm infants with PTX show morbidities such as intraventricular hemorrhage and long-term neurodevelopmental disabilities more frequently.[5]

Management of PTX includes expectant observation (EO), needle aspiration (NA), and chest tube drainage (CTD). We still do not have a consensus for the optimal management strategy in neonates.[6] In older children and adults, CTD is widely viewed as definitive treatment of PTX that allows continuous drainage. However, it is invasive, and may be complicated by injury to thoracic and abdominal organs, permanent disfigurement, and phrenic nerve injury.[7,8] Needle aspiration is an attractive alternative as it is less invasive, easier/faster to perform, and does not require prolonged placement of a foreign material. However, it is viewed as a temporizing procedure with a risk of complications related to re-accumulation of free air and the development of tension pneumothorax, respiratory failure, and cardiovascular collapse.[3,9] Expectant observation has been an underreported but a widely used treatment option in neonates. For most clinicians, the comfort with EO is confounded by lack of evidence.

This study was designed to describe our experience with the three different management strategies for this potentially serious and common neonatal condition. More specifically, we wanted to determine the safety and efficacy of EO and NA compared with CTD. Patient and pneumothorax characteristics associated with each

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of these three treatment modalities were examined. This study is expected to provide further information to assist clinicians in their management of neonatal PTX.

Materials and Methods

This is a retrospective study of admissions to our level 3 NICU from January 2017 to June 2019 who developed a PTX. Eligible patients were identified from unit-based quality improvement metrics which routinely track all PTX. Maternal and neonatal baseline characteristics and outcomes were abstracted from the electronic medical records. Respiratory support was categorized into three groups: (i) invasive mechanical positive pressure; (ii) noninvasive positive pressure [nasal intermittent ventilation and continuous positive airway pressure (CPAP)]; and (iii) support with a low-flow nasal cannula with minimal oxygen supplementation or observation in room air. Pneumothorax was defined by the accumulation of air in the pleural space on chest radiograph (CXR) as interpreted by a radiologist. The size of the PTX was determined by the degree of lung collapse: (a) collapse $\leq$ 20% was defined as small; (b) 21–39% as moderate; and (c) $\geq$ 40% as large. Safety was defined by the lack of tension (no mediastinal shift) seen on repeat CXR, cardio-respiratory stability (no changes in blood pressures/urine output needing fluid boluses or vasopressors), and no altered respiratory stability. The success of the intervention was evaluated based on the first intervention for PTX; efficacy was defined as improvement/resolution of the PTX without a need for secondary intervention(s) within 24 hours.

In the DR, the decision to provide CPAP and/or positive pressure ventilation (PPV) was made by a team leader (a neonatologist, neonatology fellow, nurse practitioner, or a physician assistant). The American Academy of Pediatrics Neonatal Resuscitation Program guidelines were utilized with initiation of CPAP/PPV for persistent apnea, gasping, or heart rate (HR) < 100 beats per minute (bpm).[10] Newborns with a HR > 100 bpm with labored breathing or cyanosis were provided supplemental oxygen and evaluated for CPAP. Pulse oximetry was used for monitoring all infants. CPAP was initiated with 5 cm of water pressure, provided via a T-piece resuscitator/face mask. Gestational age was assigned by the best obstetrical estimate. This study conformed with the Declaration of Helsinki and was approved by the Institutional Review Board.

Statistical Analysis

Continuous variables were summarized using mean and standard deviation (SD) for normally distributed data, and median and interquartile range (IQR) for non-normally distributed data. Normality was assessed using the Shapiro–Wilk test and visualized with Q–Q plots. Categorical variables were summarized using counts and percentages. Associations between medical intervention or intervention success and patient/PTX characteristics were assessed using the appropriate statistical tests: Student's two-sided *t*-test or Welch's *t*-test for normally-distributed continuous parametric variables and one-way analysis of variance (ANOVA) for comparisons of >2 such groups. The Homoscedasticity for ANOVA was assessed using Levene's test. Wilcoxon rank-sum test was used for non-parametric variables and a Chi-square test for categorical variables. Categories with expected frequencies <5% were noted and excluded from further analysis. A *p*-value significance threshold of 0.05 was used for all tests.

Significant associations were followed by pairwise group comparisons using the false discovery rate (FDR) method to control for multiple hypothesis testing. When significant differences were found, the Cochran–Mantel–Haenszel test was used to examine the effects of potential confounding variables such as gestational age and pneumothorax size. Logistic regression was used for multivariate modeling to understand the relationship between medical intervention or intervention success and confounding variables. All statistical analyses were performed using JMP®, Version 17.2 (SAS Institute Inc., Cary, NC, 1989–2024).

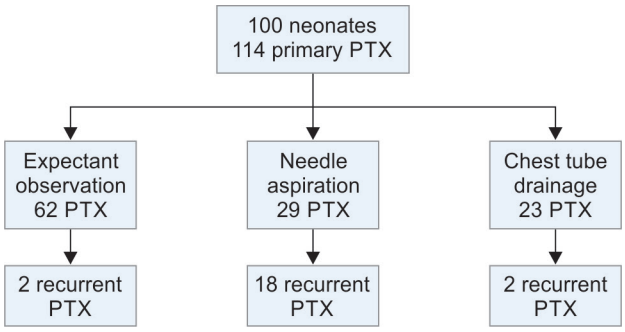

Fig. 1: Primary treatment modality and recurrences

Results

During the study period, there were 17,224 live births of which 2,408 infants were admitted to the neonatal intensive care unit (NICU). There were 114 PTXs in 100 neonates (5% of NICU admissions, 0.7% of all live births) during the study period (Fig. 1). The study population was composed primarily of late preterm and term neonates (median GA 37 weeks, IQR 34–39 weeks). Most were born by Cesarean section (C-section). Among those with PTX, 20.2% were treated with a CTD, 25.4% with NA, and 54.4% with EO. Neonates treated with CTD and NA were less mature and smaller than those treated with EO ($p < 0.001$, Table 1). More males were treated with CTD and EO than NA ($p = 0.04$). There were no additional associations between neonatal/maternal characteristics and treatment modalities. Of note, infants who developed PTX required positive pressures frequently in the DR; 53.7% received CPAP and 13% PPV (Table 1). Most neonates with PTX were admitted to the NICU (83.3%).

In our cohort, there were 57 early and 57 late PTXs. EO was used significantly more frequently for early and small PTX (Table 2 and Fig. 2). Infants with moderate PTX were more likely to be treated with NA and CTD than EO (59.3 vs 40.9 vs 13.1%, $p < 0.001$). A similar pattern was seen with late PTX (75.9 vs 78.3 vs 27.4%, $p < 0.001$), and for tension PTX (41.4 vs 39.1 vs 1.6%, $p < 0.001$; Table 2). Noninvasive PPV was the most common form of respiratory support when the initial PTX occurred. CTD was performed more frequently than EM (43.5 vs 8.1%, $p = 0.001$) for neonates on invasive mechanical ventilation while NA and EM were performed more frequently than CTD (69.0 vs 72.6 vs 43.5%, $p = 0.04$, respectively) (Table 2) for neonates on noninvasive PPV.

The efficacy of CTD was 91.3%, NA was 37.9%, and EO was 96.8%. All failed primary interventions were seen in infants with late PTX. In univariate analyses, NA, tension, size, and timing were significant factors impacting success of the primary intervention (Table 3). In multivariate analysis, only NA remained significant [adjusted odds ratio (aOR) 0.08, 95% confidence ratio 0.02–0.4]. Regarding safety, none of the neonates in our study experienced adverse events such as secondary tension PTX, cardio-respiratory decompensation, or mortality.

Table 1: Maternal and neonatal characteristics

Maternal characteristics	*Chest tube (n = 20)*	*Needle aspiration (n = 26)*	*Expectant observation (n = 54)*	*Overall (n = 100)*	*p-value*
Maternal age (years, mean ± standard deviation)	32 ± 5	32 ± 6	31 ± 5	31 ± 5	0.4
Twin gestation (*n*)	4 (20%)	0	1 (1.9%)	5 (5%)	*
Prolonged rupture (*n*)	2 (10.5%)	0	7 (13%)	9 (9.2%)	*
Oligohydramnios (%)	3 (15)	2 (8)	7 (13%)	12 (12.1%)	0.7
Neonatal characteristics	*Chest tube (n = 23)*	*Needle aspiration (n = 29)*	*Expectant observation (n = 62)*	*Overall (n = 114)*	*p-value*
Gestational age (weeks, median [IQR] ŦŦ	34 (28, 37)	36 (31, 38)	39 (36, 40)	37 (34, 39)	<0.001
Birth weight (grams, median [IQR]) ŦŦ	2205 (1010, 3325)	2675.0 (1650, 3095)	3185.0 (2886.3, 3548.8)	2932.5 (2198.8, 3391)	<0.001
Sex (% male) ŦŦ	17 (73.9%)	15 (51.7%)	48 (77.4%)	80 (70.2%)	0.04
Mode of delivery (% cesarean section)	12 (52.2%)	21 (72.4%)	35 (56.4%)	68 (59.6%)	0.25
Delivery room resuscitation					
Delivery room CPAP	9 (42.9%)	14 (50.0%)	35 (59.3%)	58 (53.7%)	0.39
Delivery room PPV	4 (19.1%)	4 (14.3%)	6 (10.2%)	14 (13%)	*
1 minute APGAR <7	5 (21.7%)	5 (17.2%)	14 (22.6%)	24 (21.1%)	0.84
5 minutes APGAR <7	4 (17.4%)	2 (6.9%)	6 (9.7%)	12 (10.5%)	*

**p*-value not reported as assumptions of Chi-square test were not satisfied. ŦSignificance driven by difference in expectant observation compared with needle aspiration and chest tube drainage groups. ŦŦSignificance driven by difference in needle aspiration compared with chest tube drainage and expectant observation groups. CPAP, continuous positive airway pressure; PPV, positive pressure ventilation

Table 2: Primary intervention and pneumothorax characteristics

Respiratory support/PTX characteristics	*Chest tube (n = 23)*	*Needle aspiration (n = 29)*	*Expectant observation (n = 62)*	*Overall (n = 114)*	*p-value*
Invasive positive pressure ventilation Ŧ	10 (43.5%)	7 (24.1%)	5 (8.1%)	22 (19.3%)	0.001
Noninvasive positive pressure ŦŦ	10 (43.5%)	20 (69%)	45 (72.6%)	75 (65.8%)	0.04
Nasal cannula/room air	3 (13%)	2 (6.9%)	12 (19.4%)	17 (14.9%)	*
Tension ŦŦŦ	9 (39.1%)	12 (41.4%)	1 (1.6%)	22 (19.3%)	<0.001
Early PTX ŦŦŦŦ	5 (21.7%)	7 (24.1%)	45 (72.6%)	57 (50%)	<0.001
Late PTX ŦŦŦŦ	18 (78.3%)	22 (75.9%)	17 (27.4%)	57 (50%)	<0.001
Size – Small ŦŦŦ	4 (18.2%)	4 (14.8%)	52 (85.3%)	60 (54.6%)	<0.001
Size – Moderate ŦŦŦ	9 (40.9%)	16 (59.3%)	8 (13.1%)	33 (30%)	<0.001
Size – Large	9 (40.9%)	7 (25.9%)	1 (1.6%)	17 (15.5%)	*

**p*-value not reported as assumptions of Chi-square test were not satisfied. ŦSignificance driven by difference in expectant observation and chest tube drainage groups. ŦŦSignificance driven by difference in chest tube drainage compared to needle aspiration and expectant observation groups. ŦŦŦSignificance driven by difference in expectant observation compared with needle aspiration and chest tube drainage groups. ŦŦŦŦSignificance driven by difference in expectant observation compared with needle aspiration and chest tube drainage groups

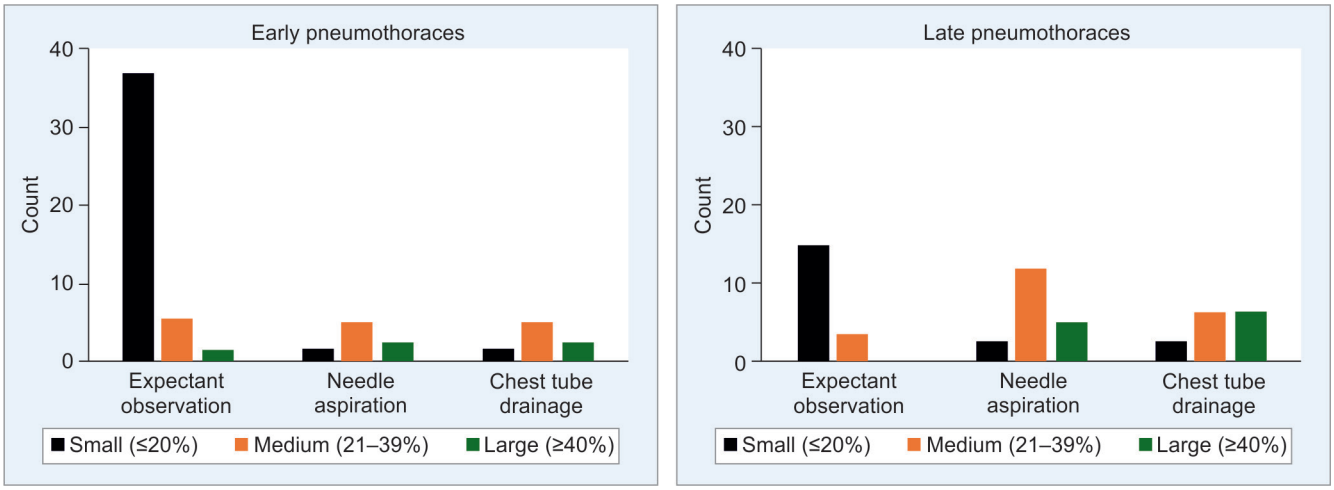

Fig. 2: Primary treatment modality, pneumothorax size, and timing. Early pneumothoraces occurred before 6 hours, late pneumothoraces at ≥6 hours of life

Table 3: Univariate and multivariate regression for factors predicting treatment success

	Crude			*Adjusted*		
Factors examined for treatment success	*OR*	*95% CI*		*OR*	*95% CI*	
Chest tube drainage (ref: Expectant observation)	0.51	0.13	2.17	0.78	0.12	4.92
Needle aspiration (ref: Expectant observation)	0.05*	0.01	0.14	0.08**	0.02	0.40
Tension component (ref: No)	0.20*	0.07	0.54	0.36	0.09	1.46
PTX size (ref: Small)	0.23*	0.09	0.57	0.94	0.21	4.24
Respiratory support (ref: Room air/nasal cannula)	0.84	0.22	2.63	1.25	0.26	6.02
Pneumothorax (ref: Early)	0.37*	0.15	0.88	0.75	0.21	2.64
Gestational Age (ref: ≥ 37 weeks)	0.65	0.28	1.5	1.39	0.38	2.64

N = 110, CI, confidence interval; OR, odds ratio. Tension component: 0 = No 1 = Yes, Size: 0 = Small 1 = Moderate/Large, Respiratory support: 0 = Room air/nasal cannula 1 = Noninvasive and invasive positive pressure, Pneumothorax: 0 = Early 1 = Late, Gestational Age 0 ≥ 37 weeks, 1 < 37 weeks.
*Statistically significant in univariate analyses. **Statistically significant in multivariate analysis

Discussion

Despite PTX occurring most frequently in the neonatal population, there is a lack of consensus among neonatologists on the most appropriate management for this condition. In this single-center retrospective chart review, we describe the three primary interventions for management of PTX, and the characteristics of patients and PTXs associated with each intervention and predictors of success. Expectant observation was most frequently practiced for small PTX diagnosed early in more mature neonates; it was safe and highly successful as a definitive intervention, not just as a temporizing procedure. The demographics and PTX characteristics of patients treated with NA and CTD were similar; they were less mature and more likely to have moderate/late PTX or PTX with tension. Needle aspiration was the definitive treatment and possibly obliviated the need for a CTD in about 40% of neonates with a PTX. Failed primary interventions occurred exclusively after 6 hours.

Like previous investigators, we found a high rate of positive pressure use in the DR and PTX. In our study, 53.7% received DR CPAP, a proportion that resembled those of Smithhart et al.[4] These investigators also detected a significant association between the need for DR CPAP in late preterm/term infants and PTX (OR 4.6, 95% CI: 3.6–6.0). The well-baby population who are at or close to term gestation frequently show asymptomatic and mildly symptomatic PTX. In contrast, the preterm/critically ill patients experience significant respiratory distress with PTX requiring respiratory support.[1,11,12] Similar to other investigators, >80% of the infants in our study were receiving noninvasive or invasive positive pressure at the time of diagnosis.[12,13]

In our cohort, most patients with PTX were term or late preterm, were male, and were born via C-section. PTX occurred in a larger proportion of preterm infants, but the total number of PTXs was larger in late preterm and term infants likely because these patients comprised a larger fraction of all infants.[3,14,15] Also, late preterm and term infants have more developed musculature, are able to generate higher negative intrapleural pressures, and have more compliant lungs. These physiologic factors predispose this population to early PTX, particularly when exposed to positive pressure. Like others, we also noted a higher incidence of PTX in male infants.[3,12,16] This finding is likely due to the increased risk for respiratory distress in male neonates; human and animal studies show less lung maturity in male fetuses.[17–21] The risk following C-section is likely secondary to retained fetal lung fluid and delayed surfactant secretion. This higher risk has been described in both elective vs emergency C-sections and in preterm and term neonates.[20,22]

Several studies have described the rate of PTX in NICU populations and among all live births. A retrospective analysis

of >71,000 NICU admissions in the Canadian Neonatal network identified a high rate of PTX with a bimodal distribution in gestational age (GA) (4% for GA < 32 weeks, 2.6% for 32–36 weeks, and 6.7% for GA ≥ 37 weeks).[2] A US study of about 13,800 live births noted PTX in 0.27% in infants born with a birth weight ≥2,500 gm and 2.5% in <2,500 gm.[12] A Swedish study of 24,000 live births reported PTX in 0.31% with 87% of PTX occurring in term and post-term newborns.[15] In our study, the overall rate of PTX was 5% which closely aligns with the rate of 4.45% in the Canadian neonatal network with a majority of PTX identified in infants with GA > 32 weeks (83%).

Historically and contemporarily, PTX has been associated with significant morbidity and mortality.[2,12] To minimize the risk for catastrophic deterioration associated with symptomatic PTX, CTD is a common invasive intervention used by clinicians.[3,23,24] This intervention offers the advantage of definitively and continuously evacuating air. However, CTD is more invasive with higher risk of injury to surrounding structures, future breast deformity, and infectious complications.[7,8] Currently, there are limited data to guide clinicians when less invasive options such as NA and EO are suitable options.[6]

Interestingly, EO was the most frequently utilized and successful intervention in our study. Previous studies examining EO have been limited to select populations or do not examine the success of EO as the primary management strategy.[14,24–26] Based on our experience, EO is highly successful in the management of early and small PTXs even for symptomatic infants and should be considered in this population. For NA, there have been two small randomized controlled trials (RCTs) comparing NA and CTD.[13,27] Arda et al.[27] randomized newborns to drainage with a CTD vs drainage with an 18 gm venous catheter. The complication rate was significantly lower (5.5 vs 30.5%, $p < 0.05$) for the venous catheter group and no infant required chest tube insertion. In a RCT enrolling neonates with symptomatic PTX, NA successfully precluded the need of a CTD in 30% of subjects. Even in preterm infants ≤32 weeks, NA was efficacious and reduced the need of CTD in 18%. In the NA group, fewer infants required assisted ventilation, and no adverse events were seen with this procedure.[13] We also found that NA could successfully avoid CTD, possibly in a larger subset (40%). Of those who failed NA, no neonates experienced hemodynamic instability due to delayed CTD.

Our study is limited by several factors. For one, it is a single-center, retrospective study. Our local practice for interventions for PTX may not be generalizable to other NICUs. Furthermore, there is variability in our practice with some clinicians preferring to immediately place a chest tube after NA. This variability may have negatively biased the success of NA as a definitive treatment option. It was also limited by the sample size, particularly regarding the number of patients who failed the primary intervention. Additionally, CXRs were interpreted by multiple radiologists and there was variation in the views obtained (anterior-posterior and/or lateral decubitus). Standardized definitions of the PTX size were utilized to minimize bias, but bias could not be eliminated.

Conclusion

In this single-center study, EO was the most frequently utilized treatment modality and was highly successful in management of small early PTXs. NA was utilized in less mature neonates with more complex PTXs; it is a safe and reasonably efficacious option that avoided more invasive CTD in some neonates.

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Ethical Approval

This study conforms with the Declaration of Helsinki and was approved by the Institutional Review Board of Hackensack University Medical Center, Protocol #2023-0123. A waiver of consent was granted by the IRB.

Author Statements

Nicole Spillane, Zuzanna Michalak, Laurie Guzman, and Sabrina Malik designed the study, collected data, performed data analysis, and wrote and edited the manuscript. Tara Lozy assisted is study design, data analysis, and biostatistical support.

Data Availability

A deidentified dataset will be made available to editors, reviewers, and readers on request.

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ORIGINAL RESEARCH

Need for Cautious Adoption of American Academy of Pediatrics Guidelines for Management of Neonatal Hyperbilirubinemia in Different Parts of the World

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Abstract

Background: Early hospital discharge (<72 hours following birth) of healthy-term and near-term infants is favored to promote family care but may have increased hyperbilirubinemia-related hospital readmissions. In this study, we compared the 2009 and 2022 American Academy of Pediatrics (AAP) discharge guidelines in the United Arab Emirates (UAE) for the impact on hyperbilirubinemia-related readmissions.

Materials and methods: This was a retrospective cohort single-center study conducted in the UAE. We reviewed records from the period January 2021 to November 2021; the infants included those with a gestational age (GA) ≥35 weeks and a birth weight (BW) ≥2,500 gms and GA ≥36 weeks/BW ≥ 2,000 gms. Infants were classified into risk zones based on pre-discharge transcutaneous bilirubin (TcB) or total serum bilirubin (TSB) levels (AAP 2009 hyperbilirubinemia nomograms). We compared the 2009 and 2022 AAP discharge thresholds for the needs for follow-up and readmissions for hyperbilirubinemia.

Results: We studied 895 newborns; 672 (75%) were born at term with a mean (± standard deviation) GA of 38 ± 1.3 weeks. Most (75.3%) were classified as appropriate for GA and 637 (71%) attended the 1st follow-up as recommended. Based on the 2009 AAP guidelines, 13 (2.9%) out of 447 (70%) were low risk; 12 (6.6%) out of 183 (29%) were low-intermediate risk; and 3 out of 7 (42.9%) were high-intermediate risk. A total of 49 (5.5%) infants were readmitted to the hospital for phototherapy. Unlike in the United States, the 2022 guidelines would have recommended follow-up visits within 2 days in a larger number [579 (64.7%)] than the 2009 recommendations [308 (34.4%)] in UAE; the overall need for phototherapy would also have been higher. However, the frequency of severe hyperbilirubinemia requiring phototherapy would have remained similar. Our population did not have more specific risk factors such as scalp bleeds, ABO isoimmunization, or glucose-6-phosphate dehydrogenase deficiency for developing severe neonatal hyperbilirubinemia. We did have a high number of missed follow-up appointments.

Conclusion: In our region, the adoption of the 2022 AAP early hospital discharge guidelines may have increased the number of follow-up visits within 2 days after discharge from the hospital and the overall need of phototherapy. These guidelines need to be specifically evaluated in different ethnic groups in various parts of the world.

Keywords: American academy of pediatrics, Bilirubin, Bilirubin encephalopathy, Direct antiglobulin test, Early discharge, Follow-up timing, Infant, Kernicterus, Low intermediate-risk, Middle East, Monitoring, Nomograms, Pre-discharge bilirubin, Readmission risks, Retrospective cohort study, Rhesus incompatibility, Serum bilirubin, Transcutaneous bilirubin.

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Keypoints

1. Hyperbilirubinemia is seen frequently in neonates; those with high bilirubin levels require close monitoring to prevent bilirubin-induced neurotoxicity.
2. We evaluated the 2009 and 2022 recommendations of the American Academy of Pediatrics (AAP) for early hospital discharge (≤72 hours after birth) on the rates of readmission for hyperbilirubinemia in the United Arab Emirates.
3. This retrospective single-center study included 895 infants with gestational age (GA) ≥35 weeks and birth weight (BW) ≥2,500 gms or GA ≥ 36 weeks/BW ≥2,000 gms.
4. In our population, the relatively liberal AAP 2022 guidelines for early hospital discharge have increased the number of follow-up visits for evaluation of hyperbilirubinemia and the overall need for phototherapy. We need to review and interpret these guidelines cautiously.

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Introduction

Hyperbilirubinemia is seen frequently in neonates and requires close follow-up, particularly within the first week of life.[1,2] Failure to promptly identify and manage it may lead to irreversible bilirubin-induced neurotoxicity (kernicterus), which can sometimes be fatal.[2,3]

Risk factors for significant neonatal hyperbilirubinemia include prematurity, low birth weight (BW), obscure sites of hemorrhage such as a large cephalohematoma, family history of neonatal hyperbilirubinemia, iso-immune hemolytic anemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency.[4] These infants are more likely to require readmission and treatment with phototherapy. Glucose-6-phosphate dehydrogenase deficiency, an inherited red blood cell enzymatic deficiency, has been seen in 7.4% of all infants in the United Arab Emirates (UAE). It can cause severe hyperbilirubinemia with a high risk of kernicterus.[4–6]

In infants with hyperbilirubinemia, the prediction of a sudden and significant rise in the TSB levels remains challenging.[7,8] In recent years, we have followed the recommendations of the AAP for early discharge of newborn infants from hospitals to promote family care. Bhutani et al.[1] developed nomograms (1999) to predict the risk of neonatal hyperbilirubinemia in 2,840 healthy-term and near-term neonates eligible for discharge between 24 and 72 hours of age. The authors recommended measuring pre-discharge serum bilirubin and evaluating the associated risk factors to provide hour-specific serum bilirubin thresholds and risk zones. These guidelines were modified in 2009, incorporating additional risk factors for neurotoxicity, such as sepsis, low serum albumin, clinical instability, and iso-immune hemolytic anemia.[9] The importance of pre-discharge bilirubin screening using TSB or transcutaneous bilirubin (TcB) measurements was stressed. Specific follow-up guidelines were also provided to assess the risk of subsequent severe hyperbilirubinemia.[9] All these additions resulted in a lower threshold for initiating phototherapy.[9–12] A new set of guidelines was introduced in 2022 for managing hyperbilirubinemia in infants born at ≥35 weeks' gestation.[4] The authors defined two new calculated metrics, "delta TSB" and "delta TcB," as the difference between the bilirubin concentrations measured by biochemical or estimated by a transcutaneous method. These are useful to predict the need for phototherapy. The pre-discharge evaluation of all neonates at risk of significant hyperbilirubinemia should include gestational age (GA), risk factors for neurotoxicity, and the delta TSB or TcB to determine the interval between discharge and follow-up, as well as the need for additional bilirubin measurements.

In our region in the Middle East, these newer guidelines seem to have increased, not lowered, the likelihood of readmissions particularly when the infants were discharged within 72 hours after birth.[8,9] To the best of our knowledge, no studies in our region have neither validated the 2022 AAP follow-up recommendations nor compared these with the hour-specific bilirubin nomogram suggested in the 2009 guidelines.[4,9] In this retrospective medical record-based cohort study, we compared the 2009 AAP hour-specific nomogram of pre-discharge bilirubin levels, which is currently employed in our hospital, with the 2022 AAP delta TSB recommendations to determine whether either of these guidelines was better for predicting significant neonatal hyperbilirubinemia. To ascertain that the two cohorts were comparable, we tabulated the risk factors associated with substantial neonatal hyperbilirubinemia in the two groups.

Materials and Methods

This retrospective, medical record-based cohort single-center study was conducted between January 2021 and November 2021 in the well-baby nursery at Tawam Hospital. The hospital is one of the largest tertiary teaching hospitals in the UAE, with ~ 2,300 deliveries annually. All necessary hospital/institutional review board permissions for the study were obtained before the initiation of the study (reference: KD/AJ/877).

We have been using the 2009 AAP guidelines in our nursery; the universal TcB screening is performed on all newborn infants at 18 hours of age. If the TcB levels were recognized as high based on the 2009 AAP hour-specific bilirubin nomogram or if specifically requested by the clinicians, TSB measurements were obtained. The last TSB levels prior to discharge were compared based on the 2009 AAP and the 2022 AAP thresholds. For this study, we defined "significant hyperbilirubinemia" when there was an indication for readmission to the hospital for phototherapy or exchange transfusion.

Inclusion Criteria

- Gestational age ≥35 weeks with BW ≥2,500 gms.
- Gestational age ≥36 weeks with BW ≥2,000 gms.

Exclusion Criteria

- Requirement of phototherapy during the initial newborn admission.
- Transfer to the neonatal intensive care unit (NICU) for any medical or surgical conditions.
- Neonates with major or multiple congenital anomalies.

Data Collection

The data were retrieved from the electronic medical records of all neonates born during the study period. These included demographic details, such as gestational age, BW, and the last TSB or TcB levels prior to discharge. Transcutaneous bilirubin measurements were performed using the JM instruments (Draeger, Inc.). The blood sample was obtained by a heel stick performed by an experienced laboratory technician or nurse, and serum bilirubin concentrations were measured using the standard direct spectrophotometry.

To make clinical decisions, we compared the bilirubin levels with the established risk zones in the 2009 AAP guidelines. In addition, we also computed the delta TSB as recommended by the 2022 AAP guidelines to assess the variance between the bilirubin level at discharge and the phototherapy thresholds. The follow-up strategies were defined and compared per the 2009 and 2022 AAP guidelines.[4,9] Infants who attended the follow-up clinic visits within the recommended time frame or earlier were monitored for the development of severe hyperbilirubinemia. We also evaluated the risk factors associated with the development of severe hyperbilirubinemia.

Continuous data were reported as mean ± standard deviation (SD). Nominal variables were reported as numbers and percentages and were compared using the Pearson Chi-square test or the Fisher's exact test for small values. All statistical analyses were performed using the Stata 17 software (StataCorp, Texas, USA), and a two-sided *p*-value was used to define statistical significance.

Results

In the study period from January 1, 2021 to November 21, 2021, a total of 2,110 neonates with a GA ≥35 weeks and a BW >2,000 gms were born at Tawam Hospital**.** Of these, 1,063 were excluded based on pre-defined criteria. Although the remaining 1,047 neonates met the inclusion criteria, 152 (14.5%) babies missed their first follow-up visit and hence, had to be excluded. All the remaining 895 infants had measurements of TcB and TSB in the hospital before discharge (Fig. 1).

Among the 895 infants enrolled (Table 1), 672 (75%) were born full-term with a GA ± SD 38 ± 1.3 weeks and a BW of

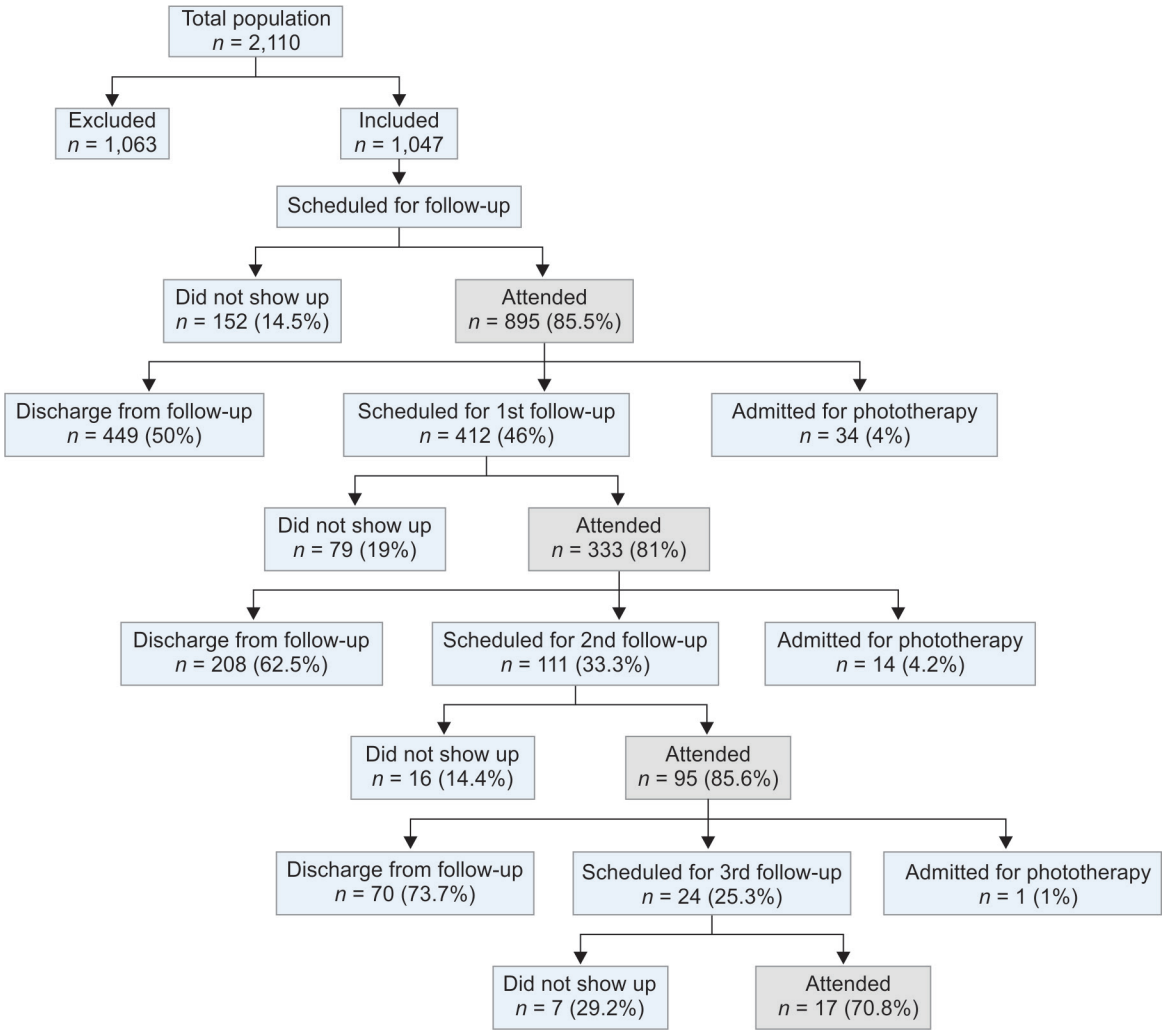

Fig. 1: Flow diagram of neonates with hyperbilirubinemia using the 2009 AAP guidelines

Table 1: Characteristics of 895 neonates with hyperbilirubinemia results expressed as number (percent) or mean ± standard deviation

Characteristics	*Results*
Gender	
Male	477 (53%)
Female	418 (47%)
Gestational age	38 ± 1.3 weeks
35–37 weeks	180 (20%)
38–40 weeks	672 (75%)
>41 weeks	43 (4.1%)
Birth weight	3,132 ± 449 gms
AGA	674 (75.3%)
SGA	204 (22.8%)
LGA	17 (1.9%)
Feeding	
Breastfeeding	805 (90%)
Formula feeding	8 (0.9%)
Mixed	82 (9.1%)
Mode of delivery	
Vaginal delivery	621 (69.4%)
Cesarean section	274 (30.6%)
Median age for the first bilirubin measurement	110 ± 60 hours

AGA, appropriate for gestational age; LGA, large for gestational age; SGA, small-for-gestational age

3,132 ± 449 gms. Four hundred and seventy-seven (53%) were males. Two hundred and four (22.8%) were small-for-gestational age (SGA). Vaginal deliveries accounted for 621 (69.4%), whereas C-sections were needed for 274 (30.6%). A large majority (805; 90%) received exclusive breastfeeding before hospital discharge. The median age for the bilirubin measurement was 110 ± 60 hours.

Out of the 895 newborns, 49 well newborns (5.5%) required readmission for phototherapy. The associated risk factors are displayed in Table 2. Small-for-gestational age status, gender, or the type of feeding did not predict readmission. Maternal diabetes

Table 2: Risk factors for readmission for phototherapy

Risk factors	*Required phototherapy (n = 49)*	*No phototherapy required (n = 846)*	*p-value*
Birth weight			0.46
AGA	31 (63%)	643 (76%)	0.22
SGA	18 (37%)	186 (22%)	0.22
LGA	0 (0%)	17 (2%)	1.0
Gestational age			
35–37 weeks	23 (47%)	157 (19%)	<0.001
38–40 weeks	26(53%)	646 (76%)	
>41 weeks	0	43 (5%)	
Gender			
Male	26 (53%)	451 (53%)	0.81
Female	23 (47%)	395 (47%)	
Mode of delivery			
Vaginal delivery	33 (67%)	588 (69%)	
Cesarean section	16 (33%)	258 (31%)	0.66
Feeding			
Breastfeeding	43 (88%)	762 (90%)	
Formula milk	0	8 (1% anker)	0.51
Mixed	6 (12%)	76 (9%)	
IDM	21 (42%)	240 (28.4%)	0.052
Cephalohematoma	2 (4%)	8 (0.9%)	0.10
ABO incompatibility	2 (4%)	61 (7.2%)	0.28
Rh incompatibility	0	18 (2.13%)	0.62
DAT positive	0	11 (1.3%)	1.00

AGA, appropriate for gestational age; DAT, direct antiglobulin test; IDM, Infant of diabetic mother; LGA, Large for gestational age; SGA, small-for-gestational age

Table 3: Outcomes of the 249 newborn infants tested for G6PD deficiency

G6PD	*Number (%)*	*Required phototherapy n = 43*	*No phototherapy required n = 206*	*p-value*
Deficiency	28 (11.2%)	2 (4.7%)	26 (12.6%)	0.18
Normal	221 (88.8%)	41 (95.3%)	180 (87.4%)	

Fisher's exact test

was present in 21 (42%) of the 49 neonates who were treated with phototherapy. None of the infants who required phototherapy had Rhesus incompatibility or a positive direct antiglobulin test (DAT).

Glucose-6-phosphate dehydrogenase testing was performed in 249 newborns (Table 3). A total of 28 babies (11.2%), all of whom were males, were identified as G6PD-deficient. Only 2 (4.7%) G6PD-deficient newborns required admission for phototherapy.

The number of infants identified to be at risk of developing severe hyperbilirubinemia before discharge using the risk zones outlined in the AAP 2009 guidelines are presented in Table 4 and Figure 2. The proportion of those in the high-intermediate risk zone who needed phototherapy looks large but the absolute numbers are small (3; 42.9%).

Compared with 2009, the 2022 AAP guidelines have significantly increased early (<2 days) follow-up visits (Table 5). Among the 895 neonates in our study, 637 (71%) had their 1st follow-up visits per the 2009 AAP recommendations. The median follow-up time

Table 4: Risk zones of developing hyperbilirubinemia before discharge as per 2009 AAP guidelines in our hospital results expressed as number of neonates (percentage)

Risk zones	*Total*	*Required phototherapy*
High risk	0	0
High-intermediate	7 (1%)	3 (42.9%)
Low-intermediate	183 (29%)	12 (6.6%)
Low risk	447 (70%)	13 (2.9%)
Total	637 (100%)	28 (4.4%)

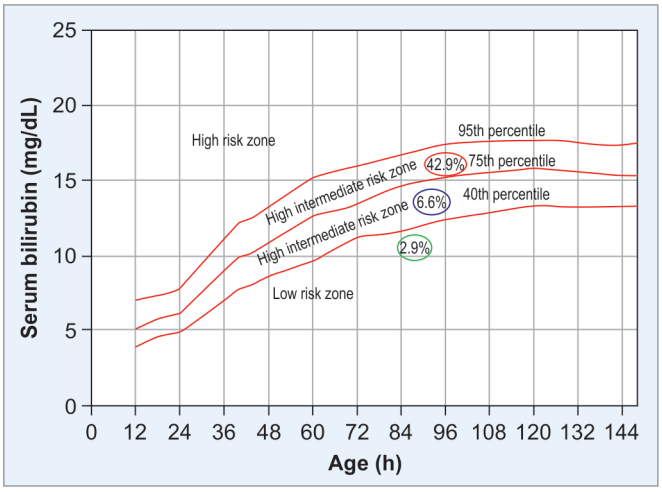

Fig. 2: Percentage of neonates in the different risk zones at risk of developing significant hyperbilirubinemia based on the risk factors (2009 AAP)

Table 5: Follow-up recommended by AAP 2009 vs 2022 guidelines

	2009 AAP		*2022 AAP*	
Timing of follow-up	*Number of infants*	*Percentage*	*Number of infants*	*Percentage*
<1 day	4	0.45%	22	2.46%
<2 days	308	34.41%	579	64.69%*
2–3 days	572	63.91%	286	31.96%*
>3 days	11	1.23%	8	0.89%
Total	895	100	895	100

*$p < 0.05$

following discharge was 104 hours (range 11–178); 28 infants (4.4%) developed severe hyperbilirubinemia. Application of the 2022 AAP recommendations has increased the number of follow-up visits and the overall need for phototherapy. The number of infants who would have been treated with phototherapy severe hyperbilirubemia was similar (Table 6).

Three hundred and thirty-three neonates were seen in the 2nd follow-up appointment. Fourteen (4.2%) were readmitted due to severe hyperbilirubinemia.

Discussion

In our region in the Middle East, neonatal hyperbilirubinemia seems to occur more frequently and reaches higher levels than in the West. Adopting the Bhutani guidelines seems to have actually increased the likelihood of readmissions, particularly when the

Table 6: Development of severe hyperbilirubinemia in neonates who presented earlier or at the recommended time after hospital discharge. The numbers below compare the 2009 and 2022 guidelines

Guideline	*Number of neonates presented at 2–3 days after birth*	*Number (%) admitted for phototherapy*
2009 AAP	637	28 (4.4%)
2022 AAP	557	24 (4.3%)

infants were discharged within 72 hours after birth.[8,9] To the best of our knowledge, no studies in our region have validated the 2022 AAP follow-up recommendations or compared these with the hour-specific bilirubin nomogram suggested in the 2009 guidelines.[4,9]

In our region, the proportion of infants in the high- and low-intermediate hyperbilirubinemia zones have been higher than those reported by Bhutani et al.[4,10] In this cohort, our absolute numbers are small, but there were more neonates with pre-discharge bilirubin levels in the high-intermediate risk (42.9 vs 12.9%; phototherapy was needed in 29 vs 19.5%, respectively) and the low-intermediate zone than in the Bhutani cohort (70 vs 61.8%, respectively; need for phototherapy in 6.6% vs none). Significant hyperbilirubinemia was seen in 2.9 vs 2.2%, respectively.

We noted that late-preterm newborns were 2.5 times more likely to require phototherapy, which is consistent with previous reports and a meta-analysis that highlighted prematurity as a risk factor for neonatal hyperbilirubinemia.[13–15] This increased risk is attributed to hepatic immaturity affecting bilirubin conjugation.[13,15] However, in contrast to findings from a systematic review demonstrating that 70% of babies requiring phototherapy were male, we did not find any significant gender-based discrepancy.[14] Unlike other similar studies, ABO incompatibility, Rhesus incompatibility, or a positive DAT were not significant contributors to hyperbilirubinemia.[16]

Unlike previous reports,[14] we did not identify G6PD deficiency as a significant contributor to the development of significant hyperbilirubinemia in this cohort. Glucose-6-phosphate dehydrogenase deficiency was identified in 11.2% of our cohort, which is similar to earlier reports from our region.[17,18] In our cohort, G6PD deficiency was seen only in males, even though some female patients have been identified in previous studies; Amro et al.[18] reported a prevalence of 11.6% in males and 3.6% in females.

Nearly 90% of our newborns were exclusively breastfed on discharge, which may represent the effective education, counseling, and support to the mothers on the benefits of breastfeeding. In our study, exclusive breastfeeding was not associated with an increased risk of hyperbilirubinemia. These results contrasted with earlier studies,[18–20] which showed a higher incidence of hyperbilirubinemia in breastfed than in formula-fed infants. The use of delta TSB or TcB for monitoring as suggested in the 2022 AAP guidelines doubled the number of follow-up visits from 34 to nearly 65% of infants within the initial 48 hours.[4,9,10] However, the rates of readmission for phototherapy remained comparable.[4,9,10]

Our research does have some limitations. Although this study has been conducted in a large hospital in the UAE, it was a single-center report from a single country. Within our population, variations in health insurance schemes create difficulties in reimbursements for planned follow-up visits. Consequently, 24% of infants could not attend their initial follow-up appointments and 3% missed subsequent follow-up visits. Moreover, G6PD tests are not systematically indicated in our institution and were conducted on only 27.8% of infants; it is difficult to be certain about its prevalence and association with neonatal hyperbilirubinemia.

Conclusion

In our region in the Middle East, neonatal hyperbilirubinemia seems to occur more frequently than in the West and there is a need for further studies to identify its causes. There is a need for cautious adoption of the Bhutani guidelines in different parts of the world as it could increase the likelihood of readmissions following early discharge. Considering the higher bilirubin levels and the difficulties in follow-up, we need to be cautious in adopting the 2022 AAP guidelines in our region; further prospective studies are needed to create a tailored nomogram specific to our population. Additionally, the significant number of missed follow-up visits emphasizes the need for effective home monitoring of these infants.

Declarations

Ethics Approval

It was granted by the Institutional Review Board (reference: KD/AJ/877). Informed consent was waived as it was a retrospective anonymized medical record review.

Authors' Contribution

Dr Ola Shahrour: Contributed to the design of the work, data collection, data analysis, and interpretation, drafting of the article, critical revision of the article, and approved the final version to be published.

Dr Hassib Narchi: Contributed to the design of the work, data analysis and interpretation, drafting of the article, critical revision of the article, and approved the final version to be published.

Dr Zohra Siwij: Contributed to the design of the work, data analysis and interpretation, drafting of the article, critical revision of the article, and approved the final version to be published.

Dr Mustafa Abdullatif: Contributed to the design of the work, data analysis and interpretation, drafting of the article, critical revision of the article, and approved the final version to be published.

Dr Aiman Ben Ayad: Contributed to the drafting and critical revision of the article and approved the final version to be published.

Dr Aiman Rahmani: Contributed to the drafting of the article, critical revision of the article, and approved the final version to be published.

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Data Availability

Data availability can be made available by the corresponding author upon a reasonable request.

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ORIGINAL RESEARCH

Pulmonary Hemorrhage Management Practices in Extremely Preterm Infants: A Global Survey

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Abstract

Background: Pulmonary hemorrhage (PHEM) can be life-threatening in extremely premature infants, with only supportive treatment available. Little is known regarding specific management strategies for PHEM because of the rarity of its occurrence and significant associated mortality.

Materials and methods: A multi-institutional working group of physicians was created with the common goal of expanding knowledge about PHEM. We designed a 14-question survey around our experience and current controversies reported in the literature. The survey was circulated via neonatal listservs (MEDNAX neonatology forum, nicu99, Envision Physician Services, and the AAP Training and Early Career neonatologists' group) to capture the management strategies of various neonatologists practicing under different settings and resources. Smartphone Apps for the Global Newborn Society were also used to reach neonatal providers around the world. The data were collected in REDCap software, and statistical analysis was conducted using SPSS version 27.

Results: There were 360 responses from 73 countries. Most neonatologists (79.2%) managed PHEM without unit-based guidelines. For the management of PHEM, there was a consensus on using endotracheal (ET) epinephrine, blood products and high-frequency oscillatory ventilation after acute PHEM. More participants responded using surfactant replacement after (55.6%) rather than during (33.1%) the management of PHEM. Post PHEM, most neonatologists obtain echocardiograms (66%) and consider treatment for patent ductus arteriosus (PDA) (65%), with the majority using acetaminophen (56.4%). Comparative analysis of practices in North America and other NICUs are also reported.

Conclusions: Our study provides a global overview of experience, and opinion-based practices used in the management of PHEM and reflects on the lack of available algorithms. Creating high-quality, evidence-based guidelines is necessary to provide appropriate care and reduce heterogeneity in the management.

Keywords: Management, Neonates, Patent ductus arteriosus, Pulmonary hemorrhage, Preterm infants, Risk factors, Surfactant.

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Introduction

Pulmonary hemorrhage (PHEM) is a rare but catastrophic complication associated with prematurity.[1] Clinical criteria used to define PHEM have included a combination of findings, including, blood or blood-tinged secretions in the lower respiratory tract, clinical deterioration that prompts escalation of respiratory support, and associated radiographic findings of pulmonary edema.[2,3] However, there remains a lack of consensus for an objective definition of PHEM.

The incidence of PHEM is inversely proportional to gestational age, with an occurrence rate of 0.1% in all infants and 8–9% in extremely preterm infants.[4] No definitive treatment is available for PHEM.[5] Supportive treatment modalities used are based on anecdotal evidence and lack consensus among providers and institutions. Furthermore, limited clinical trial evidence exists regarding strategies for prevention and treatment of PHEM. As a result, care-providers have to rely on information gleaned from retrospective studies and case series.[2]

In this study, we sought to survey neonatal care-providers worldwide to better understand contemporary strategies used for preventing and treating PHEM. These results, in combination with existing data, may help shape treatment algorithms and design clinical trials for which equipment still remains.

Materials and Methods

Questionnaire Development

We created a "PHEM work group" comprising neonatologists from multiple institutions in the US and abroad to expand our understanding

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of PHEM. The group is conducting collaborative projects and scoping reviews of the literature to enhance our knowledge about different aspects of PHEM in neonates. For this study, we created a 14-question survey based on our group's experience and various management practices enlisted in the literature. The survey was created, compiled, and circulated electronically. This study was deemed exempt by the Institutional Review Board of the Methodist Healthcare System, San Antonio, Texas.

Design of Survey

Various domains of the survey included (Supplement #1 for a Complete Survey):

- Demographics – practice location, the incidence of PHEM, level of NICU services provided.
- Risk factor assessment – gestational age, time of extubation
- Management.
  - Ventilator strategies – the type of ventilator and ventilator strategies used.
  - Use of blood products – a product used.
  - Medications – prophylactic use, endotracheal (ET) route, repeat surfactant use.
  - Consideration for patent ductus arteriosus (PDA) closure – pharmacotherapy or surgical intervention if PDA present.

Survey Dissemination

The survey was distributed as an embedded link within emails or WhatsApp and responses were collected with a RedCAP database. Various international neonatal listservs were used to reach neonatologists working under different resources and settings. MEDNAX, Neonatology Forum, Nicu99 online forum, and Envision Physician Services mailing lists were used to capture the private-practice neonatologist opinions, and the American Academy of Pediatrics Training and Early Career Neonatologists' Group (AAP TeCAN) was used to assess the academic neonatologists' opinions. Smartphone Apps for the Global Newborn Society were also used to reach neonatal care-providers around the world. The survey was disseminated in November 2021 and again in August 2024, and >80% complete responses obtained within a 4-week period of dissemination were used in data analysis.

Data Analysis

Statistical analyses were conducted using SPSS software version 27. Descriptive statistics were reported as frequency and percentage. Comparisons were performed using unpaired *t*-tests or Chi-square tests as appropriate. Statistical significance was reported at *p*-values < 0.05.

Results

Study Participant Characteristics (Table 1)

A total of 360 complete responses were received in two rounds of questionnaire responses solicited via emails and WhatsApp® messages. All responses were gathered via online responses collected in a RedCap® database. Most respondents were from North America (53.3%), followed by Australia and Asia (24.7%). More than 90% of providers reported practicing at a level III or a higher NICU (63.9% at Level III; 31.7% at Level IV). Less than 60% of participants had 4 or fewer cases of PHEM annually, and the occurrence of more than 10 cases per year was reported from 11% of the units.

Table 1: Demographics of survey participants (total 360 responders)

Demographic variable	*N (%)*
Practice location	
North America	192 (53.3)
Australia-Asia	89 (24.7)
Europe	48 (13.3)
South and Central America	16 (4.4)
Africa	13 (3.6)
Unmarked	2
Level of NICU	
Level I	3 (0.8)
Level II	8 (2.2)
Level III	230 (63.9)
Level IV	114 (31.7)
Unmarked	5
Incidence of PHEM at their practicing site	
None	37 (10.3)
1–4	213 (59.2)
5–9	68 (18.8)
≥10	40 (11.1)
Unmarked	1

PHEM, pulmonary hemorrhage

Table 2: Risk factors considered to be important for PHEM by ($N = 360$) survey responders

Gestational age as a risk factor	*N (%)*
<25 weeks	212 (58.9)
26–28 weeks	94 (26.1)
29–30 weeks	23 (6.4)
>30 weeks	27 (7.5)
Unanswered	4
Selection for PHEM occurrence scenarios (from 4 choices)	*Top selection*
Presence of hemodynamically significant PDA	200
Culture-positive early onset sepsis	97
After extubation in the first week of life	64
After the second dose of surfactant	53

PHEM, pulmonary hemorrhage. The top risk scenarios of each respondent are shown in the table. Even when averaged out for second, third, and fourth choices, the same order of concern was noted.

Risk Factors for PHEM and Preventive Strategies (Table 2)

Most respondents (59.8%) considered extremely prematurity, with gestational age <25 weeks, at the highest risk for developing PHEM. Perceived risk for PHEM among respondents decreased with increasing gestational age, with very few considering gestational age beyond 28 weeks as a risk factor. When queried regarding the scenarios that make them most worried about PHEM, of the 4 scenarios offered, the situation considered most at-risk was the presence of hemodynamically significant patent ductus arteriosus (hsPDA), followed by the presence of culture positive sepsis and the period immediately after extubation in the first week of life. The scenario with the least concern was after administering a second dose of surfactant.

Table 3: Diagnostic studies done concerning PHEM

Diagnostic modalities	*N (%)*
Clinical decline in respiratory status	323 (90)
Chest imaging	336 (93)
Hematocrit	
• ET fluid	75 (21)
• Hemogram and coagulation profile	264 (73)
Sepsis screen	160 (50)
Echocardiogram	237 (66.0)

When asked if any prophylactic management strategy was considered to prevent PHEM in the scenario of risk, only 27 (7.5%) responded in the affirmative using multiple strategies. Within those who considered prophylactic therapy, 21 used prophylactic indomethacin or ibuprofen, and 24 used high PEEP as strategies. Hemocoagulase was considered for prophylaxis by 4 respondents.

Diagnostic Modalities Used (Table 3)

For establishing the diagnosis of PHEM and initiating specific management plans, most of the respondents used the presence of blood in the upper airway along with the clinical findings of a rapid deterioration of respiratory status, which were mostly confirmed by finding diffuse changes in chest radiographs. In some situations, a drop in blood hematocrit was used as contributing evidence. Endotracheal fluid hematocrit was rarely done to establish a diagnosis. Obtaining a hemogram and coagulation profile tests were also commonly used to evaluate the need for supportive therapy. Interestingly, only about half considered doing a sepsis screen following an acute PHEM event. Most respondents performed an echocardiogram soon after the control of PHEM to evaluate the contributions of an hsPDA.

Management – During the Acute PHEM Event (Table 4)

- *Ventilator type and increase in PEEP*: High-frequency oscillatory ventilator was the preferred mode of providing respiratory support during rapid deterioration from PHEM, followed by conventional mode and high-frequency jet ventilator. The change in respiratory management during the PHEM event was one of the most consistent management strategies, with increasing PEEP to tamponade the bleeding being close to unanimous. Most respondents avoided suctioning the ETT during the event, although about a third of responders considered giving a dose of surfactant during the PHEM event.
- *Use of ET medication to stop bleeding*: Using an intra-tracheal vasoconstrictor is widely accepted. Intra-tracheal epinephrine is the most commonly used agent, but cold saline and cocaine are other less frequently considered options. Use of local coagulation agents such as hemocoagulase has also been used.
- *Transfusion of blood products*: The use of pRBCs and fresh-frozen-plasma (FFP) were the most common strategies used for hemodynamic and coagulation support during the bleeding event. Platelets were also commonly used (46.9%) followed by the use of cryoprecipitate (24.2%) to help with hemostasis. The use of recombinant factor VII a way less common, although a few centers used this product as the first line of therapy for PHEM.

Table 4: Treatment strategies for PHEM used by study responders (*N* = 360)

Immediate treatment during PHEM event	
Mode of ventilatory support	
High-frequency oscillator ventilator (HFOV)	197 (54.7)
Conventional Ventilator	122 (33.9)
High-frequency jet ventilator (HFJV)	38 (10.6)
Respiratory support	
Increasing PEEP	348 (96.7)
Avoid ET suctioning	256 (71.1)
Using surfactant during PHEM	119 (33.1)
ET administration of drugs	
Epinephrine	267 (74.2)
Cold saline	71 (11.8)
Hemocoagulase	14 (3.9)
Cocaine	6 (1.7)
Blood products	
Packed red blood cells (pRBCs)	305 (84.7)
FFP	259 (71.9)
Platelets	169 (46.9)
Cryoprecipitate	87 (24.2)
Recombinant factor VIIa	26 (7.2)
Treatment strategies (Post PHEM event)	
Repeating surfactant after PHEM	200 (55.6)
Attempting PDA closure after PHEM	234 (65.0)
PDA treatment	
Indomethacin	70 (19.4)
Ibuprofen	116 (32.2)
Acetaminophen	203 (56.4)
Surgical ligation	42 (11.7)
Transcatheter device closure	45 (12.5)
Availability of unit-based clinical practice guidelines for PHEM	75 (20.8)

FFP, fresh frozen plasma; PDA, patent ductus arteriosus; PEEP, positive end-expiratory pressure; PHEM, pulmonary hemorrhage

Management – Post PHEM Event

Since PDA is one of the common risk factors identified to be associated with PHEM, the respondents were surveyed regarding evaluating for PDA post-PHEM event. About 2/3rd of all respondents reported obtaining a screening echocardiogram after the event. Most respondents would consider PDA closure after acute PHEM, with acetaminophen being the most commonly used drug (56.4%). Some centers would consider surgical ligation or trans catheter closure of a PDA after PHEM.

Open-ended Questions in the Survey

At the end of our survey, we had a few open-ended questions to capture further comments from the respondents. Approximately 10% of the individuals commented on highlighting the practices not covered in the survey questions. The majority supported fluid restriction in the first week of life to limit volume overload, considering it a risk factor leading to PHEM. Few respondents reported obtaining a head ultrasound after the hemorrhagic event. Controversy regarding the use of surfactants in the management of PHEM was underlined in these comments, where some supported its use to control hypoxemia. In contrast, others questioned if PHEM

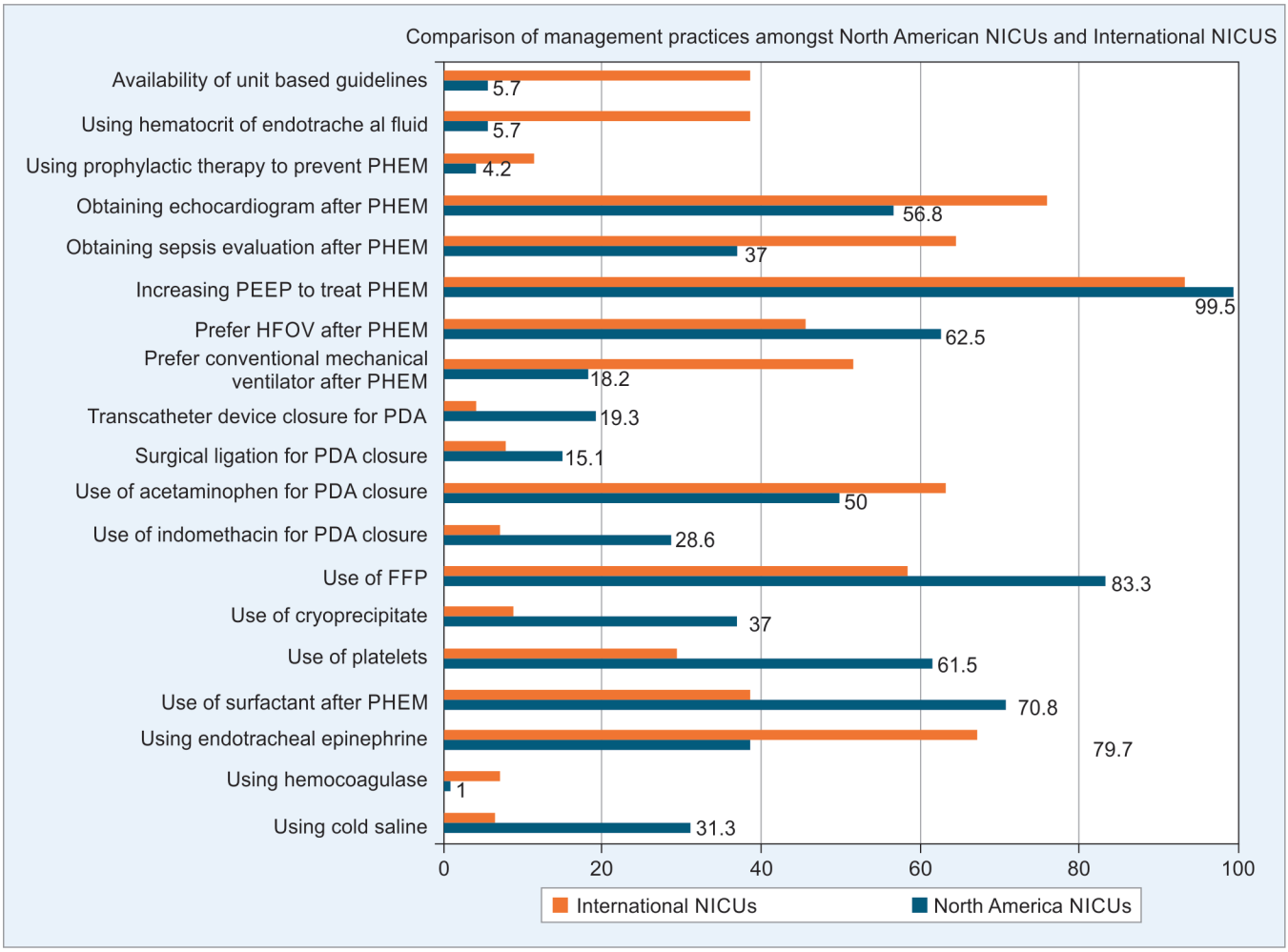

Fig. 1: Management practices in North America vs International NICUs for acute PHEM and post-PHEM * ($N = 358$)
FFP, fresh frozen plasma; HFOV, high-frequency oscillatory ventilator; PDA, patent ductus arteriosus; PEEP, positive end expiratory pressure; PHEM, pulmonary hemorrhage. *All values are statistically significant with $p < 0.05$

was the consequence of surfactant use. Interestingly, one provider reported their practice of performing bronchoalveolar lavage with diluted surfactant followed by administration of surfactant bolus.

Variation in Practices based on Location – North America vs International NICUs

When responses were stratified based on practice location, comparing centers in North America and other parts of the world, some striking differences were noted (Fig. 1). These differences in the management of PHEM were statistically significant ($p < 0.05$). Though most of the respondents were from North America ($n = 192$, 53.3%), other centers also collectively contributed significantly ($n = 166$, 46.3%). Two respondents did not comment on their site of practice and were excluded from this analysis. More centers outside North America reported having access to unit-based guidelines (38.6 vs 5.7%). Cumulative use of blood products was significantly lower in the units outside North America (FFP 58.4 vs 83.3%; cryoprecipitate 9% vs 37%; platelets 29.5 vs 61.5%). Providers outside North America were more likely to screen for sepsis (64.5 vs 37%) and PDA (75.9 vs 56.8%) after an acute PHEM event. Interestingly, providers in other units outside North America were less likely to consider PDA treatment despite increased echocardiography use for screening for PDA after PHEM.

Discussion

In this work, we sought to better understand contemporary prevention and management strategies for PHEM in neonates by surveying practicing neonatologists and neonatal trainees. Despite a lack of clinical trials focusing on this high mortality condition, we found some areas of consensus in management. These included use of high-frequency ventilation, escalating PEEP, ET epinephrine, and the use of packed red blood cells and fresh frozen plasma. However, there were many important differences in how this condition is approached within vs outside to North America.

The incidence of PHEM varies in different regions of the world, and the regional incidence and availability of resources may impact the management strategies used. Reports from Asia show a relatively high incidence of PHEM and a more aggressive management strategy.[6–10] In our study, we found that prophylaxis was significantly more common used by non-North American responders. Some centers have shown effectiveness of using

hemocoagulase as prophylaxis and treatment in lowering the incidence, duration and mortality associated with PHEM.[11,12]

We found broad agreement that extremely low gestational age at birth (<25 weeks) and the presence of a hemodynamically significant PDA were important risk factors. However, there was less agreement on the importance of sepsis and the role of surfactant therapy or post-extubation changes in alveolar pressure in the genesis of PHEM. Available published literature yields no insight into these risk factors, and there is a need for further experimental studies to clarify these issues.

There appears to be confusion regarding the role of surfactant therapy in PHEM. Some consider it a risk factor, especially after the second dose of surfactant.[13–15] Others consider surfactant therapy important as part of the management of PHEM, both during the acute event and post-event in the replacement of surfactant deactivated by the presence of blood in alveolar spaces.[16] Published reports on this topic show that the period when surfactant use was in its early phases had suggested an association with PHEM, but later studies have not confirmed this finding.[14] As we have become more selective and proficient at surfactant therapy, the association with PHEM has not been an issue. Moreover, an understanding of the physiology of surfactant deactivation has led credence to its use as a therapy during and after PHEM.[17–19] Clarification of this issue is critical if a commonly agreed upon algorithm is to be developed for the management of PHEM.

There appears to be a consensus of opinion that the diagnosis of PHEM could be established based on the following:

- Presence of blood in the lower respiratory tract (usually identified via the ET tube) in conjunction with
- An acute deterioration of respiratory and general clinical status of the infant.[20,21] The use of ET tube fluid hematocrit or the concurrent presence of coagulopathy, is not necessary for the diagnosis. However, the evaluation of hemodynamic status (blood loss) and coagulation profile (platelet and coagulation cascade dysfunction) is important in the management of the acute PHEM event. There is also general agreement among responders in our survey that the presence of a hemodynamically significant PDA should be evaluated by an echocardiogram soon after managing the acute PHEM bleed. The opinion regarding medical or interventional treatment of this PDA is, however, variable in different regions. The reluctance to treat the post-PHEM diagnosed PDA among the non-North American responders in our study is interesting.

There is evidence from the literature that systemic sepsis is one of the risk factors for PHEM.[20,21] The role that sepsis plays in the development of PHEM is not well recognized, and many responders would not routinely evaluate for sepsis after the event. Increased capillary permeability in the presence of circulating endotoxins has been suggested as one of the possible etiologies of PHEM in patients with sepsis.[7] In addition, a few case studies have shown an association between viral infections (SARS-CoV-2, cytomegalovirus and coxsackievirus) and the development of PHEM.[22–25] The risks vs benefits of screening and treating for presumed sepsis after a PHEM event may favor treatment until sepsis can be definitely ruled out.

Treatment provided for an acute PHEM event varies among responders, but there are some strategies that are almost universally applied. These near-consensus strategies could be clustered into four strategies clustered in the acronym–PHEM that stands for: (1) physical tamponade: can be achieved by endotracheal intubation or an increase in positive end-expiratory pressure (PEEP) or mean airway pressure (MAP). The most widely used method for consistently applying high pressure while limiting lung injury was the use of HFOV; (2) hemodynamic support: using blood products based on laboratory findings–mostly pRBCs or FFP or platelet transfusion, or a combination of these to replenish the losses; (3) ET medications: use of ET epinephrine or another agent that can cause vasoconstriction to limit the bleeding. The use of cold saline, hemocoagulase or cocaine intratracheally has been reported by the respondents and has some support from small reports in the literature;[11,26] (4) monitoring for risk factors, such as sepsis (sepsis work-up) and PDA (echocardiography evaluation).

Another point of controversy and variation was the use of ET suction during the event. Many responders indicated that they would avoid ET suctioning during the bleed, but at the same time some reported that they would give intra-tracheal medications or surfactant. This discrepancy may be due to the timing of the suctioning as the acute event develops. Initially, there may be reluctance to perform suction until the alveolar tamponade is established. Later, when there is a need for airway epinephrine or the instillation of another agent, suction may be needed to clear the airway and ensure that the agent reaches the site of action.[7] The construction of our question in the survey may not have been adequate to address this issue. There is also a lack of clarity in the published literature regarding this issue.

The use of blood products for hemodynamic and coagulation support also has wide consensus among the responders, with the use of pRBCs, FFP, and platelets being the most common. However, there are a not insignificant number of responders who have indicated that they would use cryoprecipitate and recombinant Factor VIIa. In 1 center, the use of Factor VIIa was part of the standard order set for an infant following PHEM. There are reports in the literature of the use of factor VIIa, but its primacy in management of PHEM in some centers but not in others is worth investigating.[5,27–29]

In summary, this questionnaire survey highlights the areas where the management of PHEM is similar among neonatologists practicing in different parts of the world. However, there are also many areas of controversy and differences in management of PHEM for which there are no clear explanations. Based on this information, we believe that there is an urgent need to develop rational guidelines based on clinical consensus.

Supplementary Material

The supplementary file is available online on the website www.jnb.org.

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REVIEW ARTICLE

Epigenetics of Down Syndrome

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Abstract

Down syndrome (DS) can show a wide clinical range in terms of type/severity of presentations/defects. This extensive heterogeneity/pleiotropy is surprising because DS is rooted in a fairly restricted genetic zone; nearly 95% have a freely segregating triplication of human chromosome 21 (*Homo sapiens* 21, Hsa21). The remaining 5% may carry translocated 21 or mosaicism of mixed clones. As in most genetic disorders, gene-dosage/copy number variations are a consideration. However, a possibility of epigenetic modifications is also being considered; this is an attractive area for study because many epigenetic marks are reversible, which might provide opportunities for therapeutic interventions aimed at prophylaxis/treatment/remission/reversal aimed at cure/rehabilitation of these patients. In this article, we have reviewed epigenetic changes in DS. Ongoing efforts show that these changes in DS might not be limited to Hsa21 but could be genome-wide; DNA methylation, post-translational histone modifications, and histone core variants have been noted. Existing data emphasize two trans-acting molecular mechanisms. The first involves enhanced expression of regulatory genes through histone modifications such as Hsa21-linked S-adenosylmethionine (SAM)-dependent methylation; and the effect of transcription factors such as Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A (DYRK1A), ETS2, high mobility group nucleosome binding domain 1 (HMGN1), Bromodomain and WD repeat domain containing 1 (BRWD1), and RUNX family transcription factor 1 (RUNX1). The second involves Hsa21q21 microRNAs (miRNAs) lethal-7c (let7c), miRNA-99a, and miRNA-125b encoded at the band q21.1; miRNA-802 at q21.12, and miRNA-155 at q21.3. Wherever possible, we focused on the protein-coding gene EURL (early undifferentiated retina and lens) as a read-out; Early undifferentiated retina and lens is the Chromosome 21 open reading frame 91 (C21ORF91) located at the centromeric boundary of the DS critical region (DSCR). We have assimilated research findings from our own laboratory with an extensive review of the literature utilizing key terms in multiple databases including PubMed, EMBASE, and Science Direct. To avoid bias in the identification of studies, keywords were short-listed *a priori* from anecdotal experience and PubMed's Medical Subject Heading (MeSH) thesaurus.

Keywords: DNA methylation, Epigenetics, Gene dosage-effect hypothesis, HDACs, Infant, MicroRNA-Let7A, Neonate, Newborn, RUNX family transcription factor 1, Trained immunity.

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Key Points

- Down syndrome (DS) is characterized by differential expression of genes located not only on chromosome 21 but also on other sites in the genome. Both altered mRNA expression and epigenetic changes have been seen.
- Epigenetic changes involving methylation have received most attention but other chemical modifications and microRNAs have also been seen.
- Many studies of DS individuals have shown extra-neuronal Aβ plaques with intraneuronal neurofibrillary tangles of hyperphosphorylated tau protein.
- Many epigenetic marks are reversible, indicating that there might be therapeutic opportunities in some clinical manifestations of DS. A new class of "epidrugs" are in development.
- Epigenetic editing is another technique in which specific epigenetic enzymes are recruited to specific genes by means of a laboratory-engineered DNA binding domain.

Introduction

Down syndrome (DS) is a leading genetic cause of morbidity and mortality. The mechanism is clearly known with triploidy of human chromosome 21 (*Homo sapiens* 21; Hsa21)-borne genetic material. These patients show a variety of congenital defects, some show organ dysfunction in the pulmonary, gastrointestinal, and renal systems beginning in early childhood, and others develop cognitive decline in later ages.[1–3] Inter-individual differences in response to therapeutic efforts are also being recognized.

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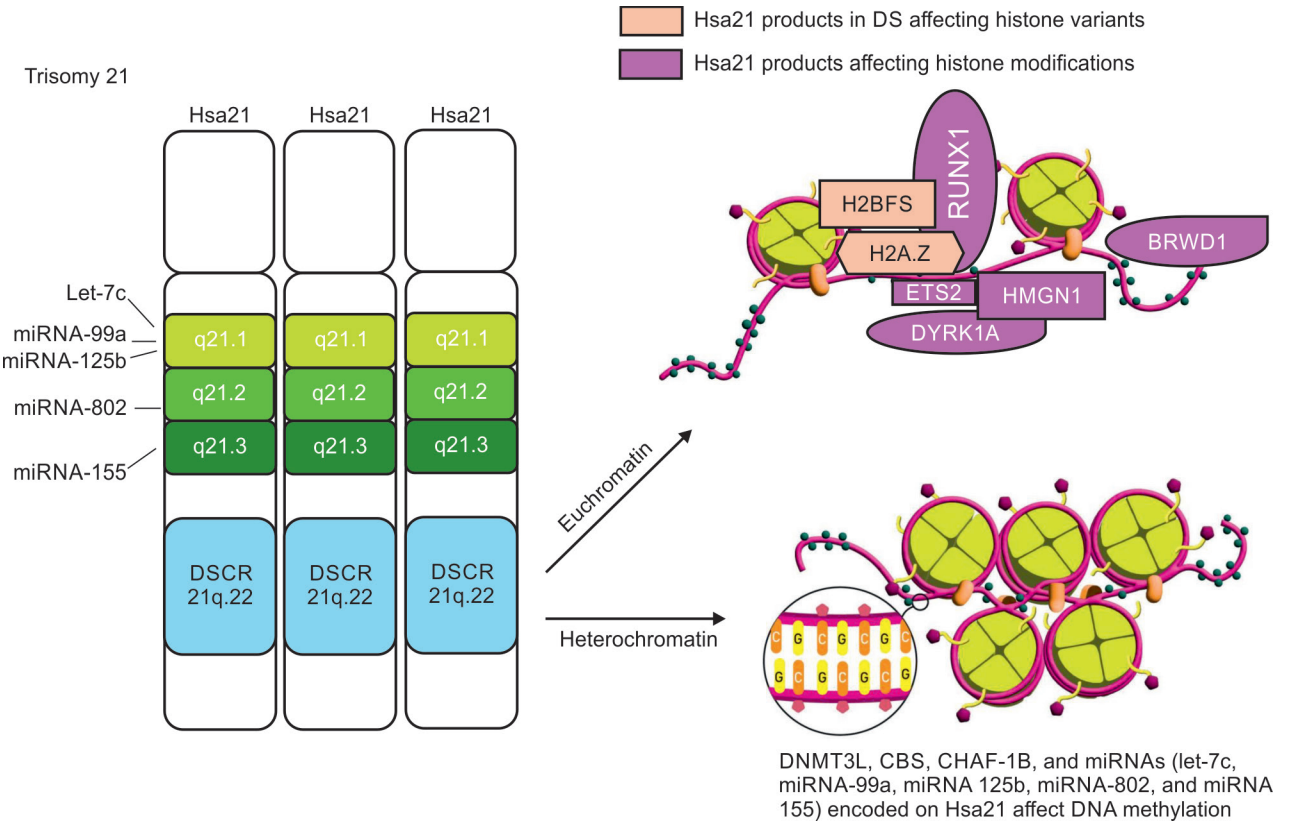

Fig. 1: Schematic overview of the genetic alterations of Hsa21 in Ts21. The long q arm of Hsa21 contains about 500 functional genes; A DS critical region (DSCR) carries most of the genes important for the DS phenotype affecting major variants and modifications in the histones. In addition to the differentially methylated regions in DNA and methylated histones, five miRNAs have also been noted to be consistently altered in Ts21: let-7c, miRNA-99a, miRNA-125b at Hsa21q21.1; miRNA-802 at Hsa21q21.2; and miRNA-155 at Hsa21q21.3

BRWD1, Bromodomain and WD Repeat Domain (tryptophan-aspartic acid (W-D) dipeptide) Containing 1; DSCR, Down Syndrome Critical Region; DYRK1A, Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A; ETS2, ETS (*Erythroblastosis Virus* E26 Oncogene Homolog 2) Proto-Oncogene 2, Transcription Factor; HMGN1, High Mobility Group Nucleosome Binding Domain 1; Hsa21, *Homo sapiens* chromosome 21; miRNA, microRNA; RUNX1: (RUNX: Runt-Related Transcription Factor 1) Family Transcription Factor 1. DNMT3L, DNA methyltransferase 3 like; CBS, Cystathionine betasynthase; CHAF-1B, Chromatin Assembly Factor 1B

The clinical heterogeneity/pleiotropy in DS is difficult to explain based solely on the relatively restricted structural genetic alterations in these patients.[4] Most, nearly 95% of these patients, show a freely segregating trisomy of Hsa21 (Fig. 1). The remaining 5% may carry translocated 21 or mosaicism of mixed clones. As we know, Hsa21 is the smallest autosomal chromosome. It is an acrocentric chromosome as its short *p* arm is disproportionally small and encodes mostly repetitive DNA that does not have a major contribution to the DS phenotype.[5] There are few satellites and hence the contribution to ribosomal RNA is also limited. The long *q* arm contains about 500 functional genes; a DS critical region (DSCR) in the distal half of the long arm (21q22) carries most of the genes important for the DS phenotype.[6–8] Within this DSCR band, a highly restricted duplicated 34 kilobase region (HR-DSCR; 21q22.13) can further account for many of the phenotypic manifestations seen in partial trisomy 21 (PT21).[8,9] This is an intergenic region between the KCNJ6-201 (potassium inwardly rectifying channel subfamily J member 6) and a DSCR transcript, the DSCR-201.[9]

To explain the high clinical variability in DS, several possible reasons have been evaluated. Gene-dosage effects are one; the triplication of Hsa21 should theoretically result in a 1.5-fold increased expression, but only 22% of the analyzed genes showed a gene-dosage effect of this level (class I/gene dosage effect). Seven percent could be grouped as class II/amplified, 56% as class III/compensated, and 15% were highly variable (class IV) in expression.[4] Most transcripts were compensated for the gene-dosage effect, where the overexpressed genes could be implicated in the DS phenotype and the highly variable genes for the phenotypic disparities.[4] Another possible explanation could be in copy number variations (CNVs) with duplications, insertions, deletions, translocations, and inversions of short or long stretches of DNA but the frequency of these genetic alterations also cannot match the observed clinical variability. Hence, there is a need for alternative explanations; one possibility is in epigenetic dysregulation of gene expression.[10] This is a potentially exciting area for research because novel instances of epigenetic "cross-talk" with various parts of the genome beyond the genes in Hsa21 are being continuously detected. These findings deserve further work.

Hence, this article focuses on epigenetic changes in DS.[1,11,12] As many epigenetic marks are reversible, these might represent possibilities for novel therapeutic measures aimed at prophylaxis/timely correction of emerging clinical signs/remission/curative reversal of established organ dysfunction/rehabilitation. Most of the current efforts are focused on DNA and histone modifications (Table 1). In the DNA, changes in methylation status are best known so far. For histone modifications, there are two major

Table 1: Aberrant epigenetic mechanisms in DS[13,14]

Gene/gene product	*Primary function*	*Downstream epigenetic effector*	*Epigenetic consequence*
PCDHG (Protocadherin gamma)[15]	Neural adhesion proteins, establishment and function of specific cell–cell connections in the brain, synaptogenesis, axonal growth	DNMT3	DNA methylation
CELSR (Cadherin EGF LAG seven-pass G-type receptor)[16]	Transmembrane cadherins, neurogenesis	DNMT3	DNA methylation
CPT1B (Carnitine O-palmitoyltransferase 1, muscle isoform)[16–18]	CPT1B gene encodes a mitochondrial enzyme, regulating entry of long chain fatty acids into the mitochondria	DNMT	DNA methylation, Mitochondrial dysfunction, oxidative stress
FLI1 (Friend leukemia integration 1 transcription factor)[18,19]	Transcription factor, regulator of myelopoiesis	DNMT	DNA methylation
CTCF (CCCTC-binding factor)[20]	Zinc finger protein; CTCF mutations are involved in the early stages of transient myeloproliferative disorder (TMD) to myeloid leukemia of Down syndrome (ML-DS) progression[21]	DNMT	DNA methylation
NOX5 (NADPH Oxidase 5)[16]	NOX5 enables proton channel activity and superoxide-generating NAD(P)H oxidase activity	DNMT	DNA methylation
TET (Ten-eleven translocation)[22]	TET DNA hydroxylases (TET1, TET2, and TET3) catalyze the oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), which can act as distinct epigenetic marks or represent intermediates of DNA demethylation.[23] Down-regulation of TET genes is associated with DNA hypermethylation in DS[23]	Oxidation of 5-methylcytosine (5mC) in DNA to 5-hmc. TET-mediated active DNA demethylation entails the thymine DNA glycosylase (TDG)-mediated DNA repair pathway for the removal of unnecessary oxidized 5mC[24]	DNA demethylase activity
REST (repressor element 1 silencing transcription factor)/NRSF (Neural-restrictive silencer factor	Neuron-specific gene regulators and transcriptional repressors play a key role in the development and function of the nervous system[24,25] Down syndrome cells show high expression of DYRK1A (dual specificity tyrosine phosphorylation regulated Kinase 1A) which leads to reduced REST and misregulation of neurodevelopmental genes[26]	REST-binding sites	DNA methylation
Micro RNAs			
miRNA-155[27]	MicroRNA[28]	Downregulates C/EBP (CCAAT/enhancer binding protein), a transcription factor that regulates the expression of SNX27 (Sorting nexin 27) Downregulation of MeCP2 (Methyl-CpG Binding Protein 2)	Hsa21-encoded mRNA,[29] altered endosomal protein sorting, decreased expression of glutamate receptors at the synaptic membrane DS-associated dementia and leukemia[29,30]
miRNA-802[13]		MeCP2 downregulation[13]	Glutamatergic aberrations because MeCP2 binds to the promoter of the GluR2 (glutamate receptor 2) and inhibits its expression

(Contd...)

Table 1: *(Contd...)*

Gene/gene product	*Primary function*	*Downstream epigenetic effector*	*Epigenetic consequence*
miRNA-99a[27,28]	Chromatin-modifying proteins		Modulation of the chromatin state by binding to chromatin-modifying proteins, overexpression may contribute to the neuropathology, congenital heart defects, leukemia and low rate of solid tumor development in patients with DS[28]
miRNA-125b-2[27]	Regulation of megakaryopoiesis		Oncogenic miRNA involved in the pathogenesis of megakaryoblastic leukemia observed in DS
let-7c[27]	Regulates cell cycle-related genes involved in the repression of cell proliferation pathways		Inhibits the tumor formation capacity of breast cancer stem cells, inhibit lung adenocarcinoma proliferation, pathogenesis of congenital heart disease in DS
miR-138-5p[31]	Zeste homolog 2[28]		Downregulation EZH2 (enhancer of zeste homolog 2), in hippocampus may be involved in the DS-related intellectual disability[31]
miR-nov1[32]	located on Hsa21		Unknown
miR-nov2[32]	located in DSCR region (chr21q22.2)		97 mRNA targets of miR-nov2 associated with cell growth, cell death, cellular localization, and protein transport
mir-1973 and mir-3196[33]	Overexpressed in the Hsa21 placenta		Regulates target genes involved in development of the nervous system
miR-329, miR-27b and miR-27a[34]	High levels of differential expression in DS fetuses		Pathogenesis of DS
lncRNAs[35]	Epigenetic modulator	Unknown	Unknown
HSA21 products			
DNMT (DNA methyltransferase)-3 like (3L)[36,37]	DNA methyltransferase	DNA methyltransferase (DNMT)-3A, DNMT3B	DNA methylation and histone deacetylation
Cystathionine beta-synthase (CBS)[38,39]	Homocysteine conversion	Depletion of genes with SAM (sterile alpha motif)	DNA methylation and histone deacetylation
Dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A)	Kinase	Sirtuin 1 (SIRT1; Histone Deacetylase, HDAC)[40]	Histone deacetylation
			Histone acetylation
		CREB and CBP (CREB-binding protein)/P300 (Histone Acetyltransferase P300, HAT)[41,42]	Histone modifications
		SWI/SNF complex (SWI/SNF related, matrix-associated, actin dependent Regulator of Chromatin)[43]	

(Contd...)

Table 1: *(Contd..)*

Gene/gene product	*Primary function*	*Downstream epigenetic effector*	*Epigenetic consequence*
BRWD1 (Bromodomain and WD repeat domain containing 1)	Transcription regulator	SWI/SNF complex[44]	Histone modifications
RUNX1 (Runt-related transcription factor 1)	Transcription factor	SWI/SNF complex[45]	Histone modifications
ETS (ETS proto-oncogene 2, transcription factor)-2	Transcription factor	CBP/P300 (HAT)	Histone acetylation
H2AFZP (H2A histone family, Member Z, Pseudogene)[46]	Histone variant	Unknown	Unknown
H2BFS (H2B histone family, Member S, Pseudogene)[47]	Histone variant	Unknown	Unknown
CHAF1B (chromatin assembly factor 1 subunit B)[48]	Constitutive chromatin protein	MBD1 (multiprotein complex with methyl-CpG binding domain protein 1) and HP1 (heterochromatin protein 1-alpha)	Methylation-mediated transcriptional repression
		CBP/P300 (HAT)[49]	Histone acetylation
HMGN1 (high mobility group nucleosome binding domain 1)	Constitutive chromatin protein	MECP2 (methyl-CpG binding protein 2)[50]	Activated or repressed gene transcription

trans-acting mechanisms: (a) histone modifications related to S-adenosylmethionine (SAM)-dependent methylation and altered expression of transcription factors DYRK1A (Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A), ETS2 [(Erythroblastosis Virus E26 Oncogene Homolog-1) Proto-Oncogene 2, Transcription factor], high Mobility Group Nucleosome Binding Domain 1 (HMGN1), bromodomain and WD repeat domain containing 1 (BRWD1) (Bromodomain And WD Repeat Domain Containing 1), and RUNX family transcription factor 1 (RUNX1) [(Runt-Related Transcription Factor 1) Family Transcription Factor 1]; and (b) altered expression of microRNAs (miRNAs) let-7c, miRNA-99a, miRNA-125b, miRNA-802, and miRNA-155. We have assimilated research findings from our own laboratory and reviewed the literature from multiple databases including PubMed, EMBASE, and Science Direct. To avoid bias in the identification of studies, keywords were short-listed *a priori* from PubMed's Medical Subject Heading (MeSH) thesaurus.

Epigenetic Mechanisms

Two epigenetic mechanisms are best recognized in DS so far; Deoxyribonucleic acid (DNA) methylation and histone modifications. DNA methylation is generally associated with condensation into heterochromatin and repression of gene expression. These changes are particularly relevant in the central nervous systems.[51,52] High levels of DNA methyltransferases (DNMTs) and methyl-CpG-binding proteins promote DNA methylation.[46] There are four major DNMTs with different functions: (a) DNMT1 maintains the methylation marks after DNA replication; (b) DNMT3A and 3B promote *de novo* DNA methylation;[53,54] and (c) DNMT3L, which stimulates the methylation activity of DNMT3A and 3B by direct binding but has no intrinsic methyltransferase activity and can suppress gene transcription in certain situations.[55–57] DNA replication also involves hydroxymethylation (5hmC generation), which is mediated by the ten-eleven translocation (TET) protein family, and is critical for DNA demethylation.[58,59] 5hmC generation has been associated with maintenance of neuronal plasticity.[60]

Many histone modifications are also seen. Histone-modifying enzymes establish (writers) or remove (erasers) particular histone marks. Acetylation enhances synaptic plasticity and memory formation, while histone deacetylation is associated with memory deficits.[61] Deregulated acetylation of H4K12, an epigenetic modification of the DNA packaging protein histone 4, has been related to memory impairment that was reversed by administration of histone deacetylase (HDAC) inhibitors.[62] These changes might be important for long-term memory formation (described below).

Altered DNA Methylation in DS

Compared with the general population, DS individuals with a full chromosome 21 trisomy (Ts21) show genome-wide DNA hypermethylation more frequently.[63] Down syndrome pathogenesis involves recurrent epigenetic changes in the DNA and chromatin of cells with Ts21. The number/sites of Hsa21 loci in DS with differential methylation (DS-DM) are not enriched compared with controls; rather, most methylated genes are roughly evenly distributed on all chromosomes. Differential methylation is a well-defined epigenetic response to the extra Hsa21 in DS. The cerebral cortex (and some other organs such as placenta) shows significantly more hyper- than hypo-methylated DS-DM loci, whereas blood cells show more hypo-methylated DS-DM regions.[64–66] However, there is a need for further work.

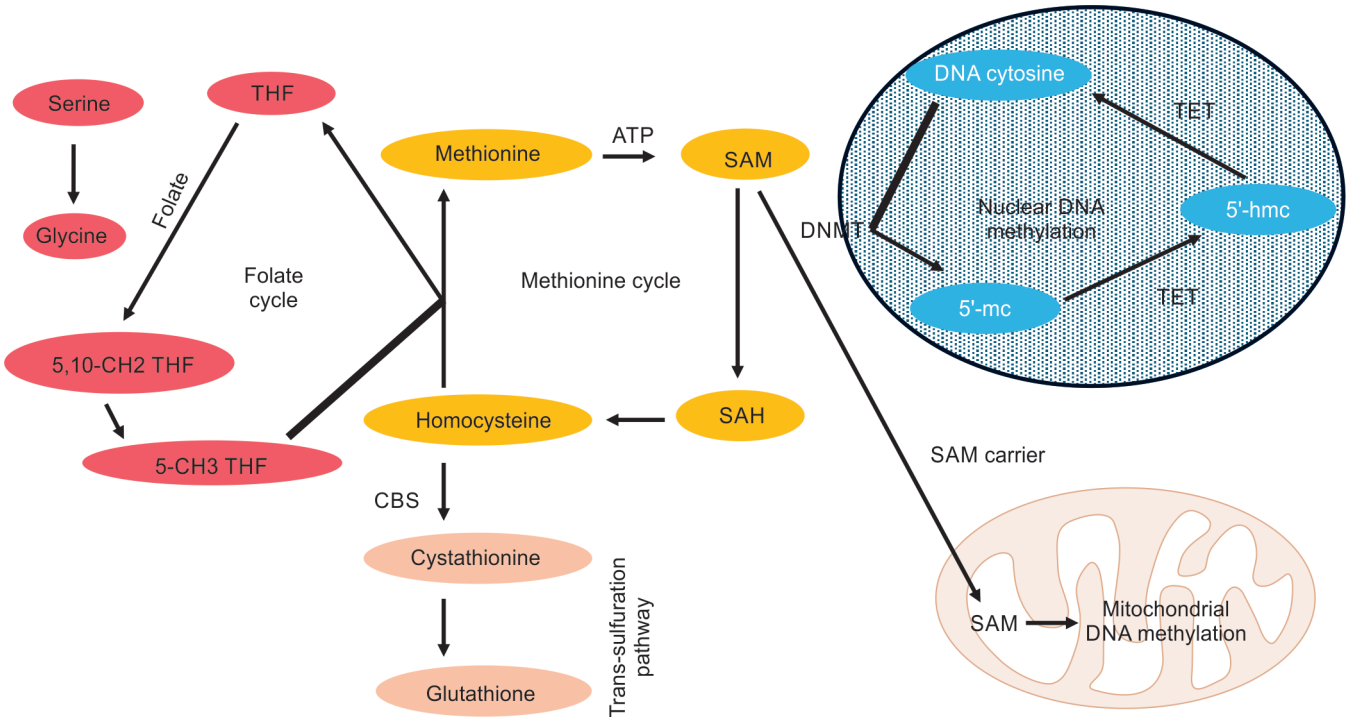

Fig. 2: Schematic overview of the relationship of DNA methylation with the methionine cycle, the transsulfuration pathway, and the folate cycle. The impact of S-adenosylmethionine (SAM) on DNA methylation in the nucleus and mitochondria has been highlighted

CBS, Cystathionine β-Synthase; DNMT, DNA methyltransferase; H2BFS, Histone H2B type F-S; H2AFZP, H2A histone family, member Z; 5′-mc: 5′-methylcytosine; 5′-hmc: 5′-hydroxymethylcytosine; SAH, S-adenosylhomocysteine; SAH, S-Adenosyl-L-homocysteine; THF, tetrahydrofolate; SAM, S-Adenosylmethionine; TET, ten-eleven translocase

In 2010, Kerkel et al.[67] performed a high-throughput screen for differentially methylated regions (DMRs) in the DS genome. Several stable, gene-specific alterations in CpG methylation patterns were notable on many autosomes other than Hsa21, indicating that an additional copy of this chromosome can alter the epigenetic patterns in other parts of the genome. Many DMRs are involved in the development and functioning of leukocytes in immune system deficiencies.[68]

Bacalini et al.[64] compared DNA methylation profiles of leukocytes obtained from 29 DS subjects with those from their mothers and non-DS siblings to identify confounding genetic and environmental factors. They noted many DMRs on Hsa21 and in other parts of the genome. These DMRs were primarily related to four functions: embryonic development, neuronal development, hematopoiesis (including RUNX1), and chromatin modulation (including TET1, and Lysine Demethylase 2B (KDM2B), encoding a histone lysine demethylase).

In DS, differential DNA methylation was first identified as a measure of cognitive functioning by Jones et al. in 2013. They examined DNA obtained from cheek swabs of 10 adult DS individuals with 10 age-matched, healthy controls. Five differentially methylated probes correlated with cognitive functioning; two were observed in TSC2 (tuberous sclerosis protein complex 2) gene, which has been associated with the tau neuropathology of Alzheimer-like cognitive deficits in DS.[69]

Down syndrome patients often show low levels of the methyl donor S-adenosylmethionine (SAM) due to overexpression of the Hsa21-encoded cystathionine β-synthase (CBS) in DS. These findings can potentially explain the low cellular methylation capacity in these patients.[38,63] Cystathionine β-synthase promotes the conversion of homocysteine to cystathionine in the synthesis of the antioxidant glutathione, lowering the availability of homocysteine for the methionine cycle (Fig. 2).[63]

Methylation-dependent downregulation of PCDHG (Protocadherin Gamma Cluster) subfamily A and B genes is expected to affect wiring processes in the developing cortex and, consequently, contribute to cognitive impairment in DS. Epigenetic dysregulation of carnitine palmitoyltransferase 1B (CPT1B) and other genes may perturb mitochondrial functions, leading to brain cell damage. Constitutive hypermethylation of PCDHG and CPT1B in brain and blood can be used as epigenetic biomarkers.

Epigenetic Marks in DS in Chromosomes Other than Hsa21

In DS, many epigenetic marks seen in autosomes other than Hsa21 seem to be more reversible. For instance, the PCDHG and CELSR3 (Cadherin EGF LAG Seven-Pass G-Type Receptor 3) genes are hypermethylated and enriched for neuron-restrictive silencing transcription factor (REST)-binding sites in DS. Protocadherins encode the largest group of the cadherin superfamily of cell–cell adhesion proteins.[70–72] The protocadherin family is subdivided into the clustered and non-clustered protocadherins, in addition to the atypical fat, dachsous (a cell adhesion molecule), and 7-transmembrane (CELSR) cadherins. The α-, β-, and γ-protocadherins constitute a 1-Mb cluster with 60 genes on chromosome 5q31.[73] The most thoroughly studied is the γ cluster, which includes 22 tandemly arranged genes. Findings suggest that constitutive hypermethylation occurs early, during embryogenesis before separation of germ layers, affecting multiple cell types and tissues.

The PCDHG and CELSR3 are interesting because these can be pharmacologically modulated by protein kinase C and calcineurin inhibitors. Similar to epigenetic drugs for cancer treatment, it may be feasible to develop epigenetic therapies for enhancing cognition and/or phenotypes in DS.[13] Ten-eleven translocation proteins hydroxylate 5-mC into 5hmC, an early intermediate of DNA demethylation. 5hmC-rich proteins are differently methylated in DS and downregulated in the DS placenta leading to hypermethylated regions.[22,64,74,75] Most of the genome-wide changes are most prominent in the neurons.

Histone Modifications in DS

Histone Core Variants

Histone core variants can also influence gene expression. Incorporation of different histone variants into the nucleosomal core affects the chromatin structure.[76] Most variants have been discovered for H2A and H3 and were reported to have a diverse role in gene expression regulation. Incorporation of macroH2A is associated with gene repression.[76,77] Hsa21 encodes the H2A histone family member Z (H2A.Z) and the H2B histone family member S (H2BFS).[46,47] H2B histone family member S was earlier considered to be a pseudogene but is now believed to encode a protein that is predicted to enable DNA binding activity and protein heterodimerization activity.[78] Deoxyribonucleic acid hypermethylation of the H2BFS gene has been reported in villus samples of DS fetuses compared with non-DS controls.[79]

Two Hsa21-encoded constitutive chromatin proteins contribute to nucleosome assembly: chromatin assembly factor 1B (CHAF1B) and HMGN1. The CHAF1B protein is involved in nucleosome assembly on to newly replicated DNA by recruiting H3 and H4.[80,81] Chromatin assembly factor 1B forms a multiprotein complex with the methyl-CpG binding protein 1 (MBD1) and heterochromatin protein 1 (HP1), which, again, demonstrates the importance of epigenetics in DS.[48] High-mobility group N1 affects posttranslational histone modifications, in particular it inhibits phosphorylation of H3S10 and H3S28 and enhances H3K14 acetylation via CBP (cAMP response element-binding protein-binding protein)/P300 HAT (histone acetyltransferase), and has been described to regulate Methyl CpG binding protein 2 (MECP2) expression.[49,50] These findings are likely important because an analogous loss-of-function mutation in CBP/P300 in Rubinstein–Taybi syndrome has been associated with intellectual disability.[41,82] Lysine methylation in histones is associated with learning-dependent synaptic plasticity and hippocampus-dependent long-term memory formation.

Methyl CpG binding protein 2 is an important factor in glutamate receptor-mediated homeostatic responses after neuronal activation.[83] It is highly expressed in the brain and can activate or repress gene transcription.[84] Altered MECP2 activity may result in intellectual disability.[50,85] Abuhatzira et al. reported transcript levels of HMGN1 to be increased with 50% and transcript levels of MECP2 to be decreased with 30% in brain tissue of DS patients compared with non-DS age-matched controls.[50] In mice, it was found that altered HMGN1 protein levels resulted in histone modifications in the MECP2 promoter and a modified chromatin structure. Therefore, overexpressed HMGN1 might disturb normal learning and memory processes through reduced MECP2 protein expression and subsequently altered epigenetic marks.

Down syndrome is marked by hypermethylation of CGIs in the histone H1 (HIST1) gene cluster comprising HIST1H4A, HIST1H3A, HIST1H2BK, HIST1H2AL, and HIST1H1B on chromosome 6.[86] The cluster constitutes 80% of genes encoding the canonical histone proteins (H2A, H2B, H3, and H4) and the linker histone (H1).[87] Differential methylation of the HIST1 gene cluster may suggest a downstream effect on chromatin structure and transcriptional dysregulation in trisomic cells. Differential methylation of 13 genes encoding zinc-finger transcription factors is also seen.

Histone Tail Modifications

Letourneau et al. studied differential gene expression in fibroblasts from monozygotic twins discordant for Ts21; they compared trisomic and disomic cells for gene expression without the noise of genomic variability.[88] Differential gene expression was distributed in specific regions of chromosomes, known as gene expression dysregulation domains (GEDDs). The observed differences in gene expression could be related to an altered chromatin state in the trisomic cells. Trisomic and disomic fibroblasts were compared for DNA methylation, particularly H3K4me3. Although differences in DNA methylation were not correlated to the GEDDs, differences in H3K4me3 profiles between the twins correlated with the GEDDs for nearly all chromosomes. Therefore, the altered H3K4me3-associated chromatin state relates to the altered gene expression in trisomic compared with disomic fibroblasts. In DS, post-translational histone modifications in five HSA21 genes, namely DYRK1A, ETS2, HMGN1, BRWD1, and RUNX1, are known to influence particular histone modifications.

MicroRNAs and Long Non-coding RNAs in DS[13]

The extra copy of Hsa21 in DS provides an additional gene dosage of all of the miRNA genes encoded on Hsa21.[46,89] The Hsa21 region q21.1–21.3 encodes 5 miRNAs; let-7c, miRNA-99a, and miRNA-125b at q21.1; miRNA-802 at q21.12 and miRNA-155 at q21.3.[27] Together, these miRNAs can target the mRNA 3′-UTRs of approximately 3,630 protein-coding genes whose dysregulation could help explain the high complexity of the DS phenotype.[27] miRNA-155 is located next to β-amyloid precursor protein (APP) gene and might regulate the expression and the generation of amyloid peptides.[90] The other 4 miRNAs, let-7c, miRNA-99a, miRNA-125b, miRNA-802, and miRNA-155 might also have some ancillary control over the expression of β-APP and regulatory secretases, cleavage enzymes, or related molecules that might alter β-APP expression and consequently, amyloidogenesis.[27] MiRNA-125b is known to be involved in neurotrophic support, synaptogenesis, neuroinflammation, innate immune signaling, and amyloidogenesis.[90] NF-κB activation during inflammation also seems to be an important transcriptional regulator of miRNA-99a, miRNA-125b, and miRNA-155.[27,90] During this process, it interacts closely with miRNA-125b and -146a in the regulation of mRNA targets such as 15-lipoxygenase, synapsin-2, complement factor H, and tetraspanin-12.

The MiR-99a is a key regulator of miRNA expression during pregnancy.[91–95] Maternal plasma miR-99a levels are altered in pregnancies with fetal congenital heart defects (CHDs).[96] It is involved in early stages of cardiomyogenesis by modulation of Nodal/Smad2 signaling; altered expression could be involved in the pathogenesis of several DS-related CHDs.[94,97] MiR-99a, along with Hsa21-derived let-7c and miR-155, is overexpressed in the heart of DS patients who have CHDs.[98,99]

The MiRNA-155 is implicated in the dysregulation of the endosomal pathway in DS pyramidal neurons.[100] It downregulates the CCAAT/enhancer binding protein (C/EBP), a transcription factor

that regulates the expression of the Sorting nexin 27 (SNX27) to promote glutamate receptor recycling from early endosomes to the (synaptic) plasma membrane.[101,102] Down syndrome brain samples show low expression of C/EBP and SNX27 and increased miRNA-155. SNX27-deleted mice show synaptic dysfunction and deficits in learning and memory, which are corrected by overexpression of SNX27.[102] *In vitro*, overexpression miRNA-155 in DS neurons altered endosomal protein sorting with decreased expression of glutamate receptors at the synaptic membrane, thereby affecting normal synaptic functioning.[102]

In DS, miRNA-155/miRNA-802-mediated downregulation of SNX27 and downregulation of MeCP2 might cause glutamatergic aberrations with impaired synaptic plasticity in the hippocampus and related learning and memory deficits.[103] Similar changes have been seen in Rett syndrome, which also manifests with developmental delay and learning/memory impairment.[50,85,104]

Kuhn et al. examined the hippocampus and heart of human fetuses with DS for the 5 Hsa21-encoded miRNAs. MiR-155 and miR-802 are aberrantly expressed in the hippocampus of a genetic mouse model of DS and human DS cortex brain tissue, impairing neurogenesis.[105] These miRNAs have been associated with cognitive impairment in DS.[106] MiR-155 is also overexpressed in peripheral tissues of DS patients.[107] These findings provide additional evidence that the five Hsa21-resident miRNAs partake in immunologic defects and neural development of DS patients. Jin et al. hypothesized that suppression of epigenetic regulators such as the TET-family genes could promote hypermethylation.[22]

Lim et al. showed differential expression of miR-1973, miR-3196, miR-504, miR-191, miR-133b, and miR-188-3p in Ts21 and control placentas.[108] However, these findings could not be recapitulated in other studies despite larger sample groups.[91,93,95,108–110]

Many recent studies have focused on maternal plasma miRNAs as biomarkers of DS; many circulating miRNAs and miRNA-containing exosomes become measurable in the late 1st trimester and can be useful for non-invasive prenatal diagnosis.[111–113] Specific markers such as miR-99a and miR-3156 levels can also be useful. Such measurements in amniotic fluid, placenta, and umbilical cord blood could be even more sensitive/specific but the need for invasive procedures to obtain these tissues limits their clinical application.

Long non-coding RNAs (lncRNAs) are involved in various regulatory roles in gene expression, including modulation of the chromatin state by binding to chromatin-modifying proteins.[35,114] A lncRNA database search revealed that many lncRNAs with yet unknown function is encoded on Hsa21.[115] The importance of lncRNAs in synaptic plasticity and learning and memory demonstrates the necessity to investigate the role of these and other lncRNAs in DS.

Studies show global hypermethylation in DS placental villi compared with controls; epigenetic changes are already present in early development.[22,79] Measurement of fetal/maternal methylation ratios in specific DMRs can help in non-invasive prenatal diagnosis of DS.[116,117]

Epigenetic Marks in Mitochondrial DNA in DS

Deoxyribonucleic acid methylation is a stable epigenetic modification though intermediate DNA modifications are present; many such marks are being continuously identified in mitochondrial DNA in DS. S-adenosyl methionine is required for methylation of cytosines in mitochondria by mitochondrial DNMT1, the only catalytically active DNMT in mitochondria.[118,119] Down syndrome may be associated with lower mitochondrial SAM levels than in controls.[38] In contrast, nuclear DNA is hypermethylated in DS.[63,64,38,120] These mitochondrial findings reflect low expression of mitochondrial enzymes and impaired adenosine triphosphate (ATP) synthesis, which result in less expression of SAM for DNA methylation.[121–123] CPT1B, a mitochondrial enzyme, regulates the entry of long chain fatty acids into the mitochondria. Similarly, methylation of two other genes has also been associated with mitochondrial dysfunction and oxidative stress in DS brains. NADPH oxidase 5 (NOX5) gene on chromosome 15 (NOX5) is a calcium-dependent protein that produces superoxide and functions as a calcium-dependent proton channel.[124] Superoxide dismutase 1 (SOD1), a key enzyme in the free radical metabolism, is overexpressed from the DSCR on Hsa21.[125] Increased production of reactive oxygen species and/or deficient antioxidant capacity contribute to brain damage and cognitive impairment in DS and cognitive impairment.[126–128]

Mitochondria are the major cellular source of high energy intermediates such as acetyl-coenzyme A, nicotinamide adenine dinucleotide (NAD^+), SAM, and ATP, which are, respectively, involved in acetylation, deacetylation, methylation, and phosphorylation of histones.[39] Aberrant mitochondrial production of these high energy intermediates in DS, for example, due to the overexpression of CBS, is likely to cause alterations in post-translational histone marks in DS. A recent study revealed that incubation of DS lymphoblasts and fibroblasts with EGCG counteracted mitochondrial dysfunction. EGCG-stimulated mitochondrial biogenesis and rescued ATP synthase catalytic activity and oxidative phosphorylation, probably restoring the levels of one or more high energy intermediates.[129] In addition to its inhibitory effect on DYRK1A, EGCG might thus improve learning and memory by rescuing mitochondrial functioning in DS.

Cell/Tissue-specific Changes in DS

Neuronal Changes

Cortical neurons in DS and its mouse models frequently show dendritic abnormalities.[130] Deoxyribonucleic acid methylation, condensation into heterochromatin, and repression of gene expression have been associated with formation of memory circuits.[51] These findings are potentially attractive because reversibility of DNA methylation in neurons might offer therapeutic opportunities for neurological/cognitive deficits in DS. Progressive neuronal cell death and disruption of neuronal network formation with reduced dendrite branching and synaptic connectivity has also been observed. In both the normal and DS brain, regional variations with the cerebral cortex showing more hyper-methylation than the cerebellar cortex are known.[65] Cortical neurons display abnormalities in the length of synaptic contact zones and synaptic density.[126,130–132] Genome-wide methylation studies have identified epigenetic signatures of DS in several tissues, including leukocytes, skin fibroblasts, buccal cells, liver, placenta, and brain.[22–65,67,69,133–135]

In DS, neuronal abnormalities are influenced by the genes DYRK1A, ETS2, HMGN1, BRWD1, and RUNX1. DYRK1A, a dual specificity tyrosine-phosphorylated kinase, has been implicated in the learning deficits.[136] These protein kinases catalyze autophosphorylation on tyrosine residues and phosphorylation of serine/threonine residues on exogenous substrates. Studies in Drosophila and mice have shown that DYRK1A is necessary for normal brain development in a dose-sensitive way.[137,138] Increased DYRK1A expression in murine cortical neurons reduced dendritic

growth and complexity.[43] Transgenic mice that overexpressed DYRK1A showed impaired cognitive flexibility and spatial learning. These findings emphasize the role of DYRK1A overexpression in DS-related intellectual disability.[139]

Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A has a dual effect on histones. It phosphorylates the threonine residue 522 of the SIRT1 HDAC, which promotes deacetylation and might contribute to deteriorating cognitive capacities.[40] It also phosphorylates the cyclic AMP response element-binding protein (CREB) at the serine residue 133, inducing the recruitment of the CBP/P300 – a histone acetyltransferase that promotes CREB-mediated expression of genes.[42,140] Besides DYRK1A, two other HSA21 proteins influence the activity of CBP/P300: ETS2, and the nucleosome-binding (HMGN1).[49] The HAT/HDAC balance is dysregulated in DS, causing aberrant histone acetylation that affect learning and memory processes.

Upregulation of DYRK1A suppresses REST mRNA levels in fetal cortex and neurospheres, which suppresses genes in close proximity to REST binding sites.[16, 24,43,141] REST regulates many neuronal genes involved in the function of ion channels, neurotransmitter receptors, and synapses.[24,142] Its expression is highest in undifferentiated progenitors and decreases with neuronal differentiation.[143] Its expression is also suppressed in neurospheres derived from fetal DS brain cells and even in DS placental villi.[22,141]

Methylation of REST-binding sites in DS may facilitate genome-wide methylation. In mouse stem cells it was shown that REST binding can induce/maintain a low methylation state.[144] Consistent with other genome-wide expression and methylation studies, many differentially-methylated CpGs on Ts21 were associated with genome-wide epigenetic dysregulation.[4,16,17,67,64,69,145]

Protocadherin Gamma Cluster hypermethylation in DS brain could be mechanistically related to cognitive impairment. These genes encode transmembrane receptors with an intracellular, a transmembrane, and an extracellular domain, which are present in most neurons, synapses, axons and dendrites.[146–148] Different PCDHG knockout mouse models suggest a role for γ-protocadherins in promoting dendritic self-avoidance, arborization, and synaptic development in cortical neurons.[147–150] Protocadherins comprise an important mechanism of cell–cell adhesion and neural circuit formation.[70–72] Protocadherins are frequently upregulated in DNMT3 knockout mice.[151] The promoters of PCDHG A and B genes are frequently methylated and these genes are underexpressed in the fetal DS cortex. In contrast, the expression of PCDHG C genes, which are ubiquitously expressed and not regulated in the same way as subfamilies A and B, remains unchanged.[151]

Cadherin EGF LAG Seven-Pass G-Type Receptor 3 (CELSR) is a key mediator of planar cell polarity (PCP). It is upregulated in the DS cortex and is also frequently hypermethylated. This increased methylation may suppress repressor activity or confer an enhancer-like activity.[152] However, unlike γ-protocadherins, the CELSR3 cell surface protein suppresses the growth and arborization of dendrites (and other types of neurites) in the developing DS cortex.[153] Although neuronal density appears to be normal, DS fetal brains are characterized by reduced dendrite branching and impaired synaptosomal structure, cortical lamination defects and dendritic spine structural anomalies.[130,131,154]

Down syndrome neurons begin to show accelerated DNA methylation in utero and this process continues into adulthood.[135] In DS, hypermethylated sites (8,624) were six times more frequent in the fetal cortex in DS than hypomethylated sites (1,447).[16] The frontal cortex in DS fetuses shows increased DNMT3L protein, and there can be higher *de novo* methylation in neuroprogenitors.[134] This hypermethylation may persist in fetal DS brain and other tissues, even though DNMT3A and DNMT3B can get downregulated.[22,133,135]

Methylation of memory suppressor genes has been identified with diminished activity of memory promoting genes.[51,52] In DS, trimethylation of H3 lysine 4 (H3K4me3) has been noted in neurons, where it can affect synaptic plasticity, learning, and memory.[88] DNMT3L lacks the catalytic domain but collaborates with the methyltransferases DNMT3A and DNMT3B.[57] These genes are expressed at higher levels in various tissues during early development than in adults.[155]

Neural Derivatives from Induced Pluripotent Stem Cells (iPSCs) as an *In vitro* Model of DS

The iPSC-derived neural model has a transcriptional profile comparable to that of fetal brains at the early and mid-gestational stages.[86,156] The chromosomal distribution of DMPs in trisomic neural cells shows a significant enrichment of hypomethylation on chromosome 21 consistent with other studies.[16,134] It has been seen that these models have a hypomethylation of chromosomes 2, 8, 19, and 22 whereas chromosomes 6 were enriched and 17 depleted with hypermethylated probes.[86] Uneven chromosomal distribution of DMPs has been observed across a variety of cells and tissues with Tsa21. [16,64,66,134] It may be caused by genomic imbalance in Tsa21 affecting the chromosomal organization in 3D, with distorted interactions between certain genomic regions, resulting in a skewing of the chromosomal and sub-chromosomal DNA methylation pattern. The differentially methylated CGIs in trisomic neural cells were preferentially hypermethylated consistent with previous studies of the frontal cortex and glia cells in DS brain specimens.[65,86,134,157] Differential methylation in HOXA3 and/or HOXD3 has previously been observed in glia cells from DS fetal brains, peripheral blood leukocytes and fibroblasts of DS patients, and undifferentiated iPSCs with Tsa21 suggesting dysregulated HOX genes may play an important role in altered development in DS.[64,65,133] Induced Pluripotent Stem Cells with Tsa21 differentiated into neural lineages may serve as a translatable model for the identification of epigenetic changes that are associated with transcriptional perturbations in DS neurogenesis.

Hematopoietic Changes

The "TF gene over-expression and binding site occupancy" hypothesis in Hsa21 suggests that this condition promotes gene expression, particularly those involved in hematopoiesis. One example is the factor RUNX; RUNX DNA-binding sites are strongly enriched in DS-DM loci in DS vs. control T lymphocytes.[65,158] In DS, higher occupancy of specific binding sites on various chromosomes and protection from methylation promotes TF-mediated gene expression.[20]

Runt-related transcription factor 1 is a crucial regulator of hematopoiesis particularly in early development.[159] It is one of the most differentially methylated genes in blood in individuals with DS.[64,66] Although DS was largely associated with hypomethylation on Hsa21, significant hypermethylation was seen at RUNX1, as reported previously in DS.[64–66,160] Runt-related transcription factor 1 hypermethylation in DS was specific to the proximal P2 promoter, which is the dominant regulator of RUNX1 expression during embryonic development, driving formation of the hemogenic

endothelium and early hematopoiesis.[161–163] Dosage of RUNX1 during these early stages is tightly controlled, suggesting that RUNX1 downregulation via P2 promoter hypermethylation may be required for viable embryo development in DS.[164,165] The distal P1 promoter becomes active once cells commit to the hematopoietic lineage and is the predominant promoter in definitive hematopoiesis, consistent with the pattern of RUNX1 expression we observed in DS FL myeloid progenitors.[162,163] Promoter switching from P2 to P1 involves changes in DNA methylation at the P1 promoter but not at P2, which was found to be unmethylated across cell types, suggesting that P2 hypermethylation is unique to DS.[166] Down syndrome-associated hypermethylation at RUNX1 also widely seen in DS brain tissue.[65,157] Runt-related transcription factor 1 plays a role in proliferation and differentiation of select neural progenitor cells, including in hippocampal precursor cells.[167,168]

The RUNX1 gene maps to Hsa21 and, despite partial gains of CpG methylation in its promoter/enhancer region, RUNX1 mRNA is modestly over-expressed in DS lymphocytes. The over-expressed RUNX1 protein is occupying its cognate DNA binding sites on various chromosomes to a greater extent, thereby blocking CpG methylation in and around these sites. Binding sites for ETS-family factors, encoded by genes on Hsa21, also showed enrichment among DS-DM loci in some tissues.[158] The "core set" of loci with strong DS-DM across multiple human tissues are significantly enriched in CTCF insulator binding sites.[65,158] Since CTCF is not encoded on Hsa21, there is an indirect link between Hsa21 and altered methylation at specific CTCF sites mediated by changes in the amounts of other DNA binding proteins that are encoded on Hsa21 and associate with CTCF on chromatin, or through CTCF binding sites being hypersensitive to altered activity of methylation pathway enzymes and cofactors, several of which are encoded on Hsa21.

The DS-DM affects hematopoietic development. Many target genes encoding signaling proteins and transcription factors have been identified, such as the Transmembrane Protein 131 (TMEM131).[64,65,67] This trans-membrane protein marks lymphocyte precursor cells. Another, the SH3 Domain Binding Protein 2 (SH3BP2), is a signaling adaptor that has been best studied in B lymphocytes. The zinc finger DHHC-type palmitoyltransferase 14 (ZDHHC14) is a palmitoyl transferase that regulates receptor tyrosine kinases. And RUNX1 is critically needed for hematopoietic stem/progenitor cell development. Many genes with DS-DM in neural cells, such as those encoding multiple protocadherin-family proteins, neuroligin-2, cytohesin-2, the signaling receptor amigo-3, and the brsk-2/sad-a kinase may also play a role in other progenitors.[65]

Muskens et al.[18] performed the largest, and first multi-ethnic, epigenome-wide association study of DS in blood cell samples at birth, confirming several known loci and identifying many novel regions, including at FLI1, that were significantly differentially methylated in newborns with DS compared with those without DS.[18] They used newborn dried bloodspots (DBS), which helped detect differentially methylated loci associated with DS, as epigenetic influences of environmental exposures and age-related changes as well as drift would be much reduced compared with studies in older individuals.

The 652 epigenome-wide significant CpGs and 1052 DMRs demonstrate the profound epigenome-wide consequences of Ts21, which likely contribute toward phenotypic variation in DS. The majority of DS-associated DNA methylation changes were found on euploid (non-21) genes, supporting that Ts21 results in genome-wide perturbations in gene regulation.[64,66,88,169,170] These results elucidate the early life, genome-wide perturbation of gene expression in hematopoietic cells that broadly correlates with differential DNA methylation patterns in DS.[18]

The overlap of DS-associated DMRs with GWAS loci for both cognitive- and hematological traits, such as at Kruppel-like transcription factor 16 (KLF16), further supports the possibility that epigenetic dysregulation may underlie both hematologic defects and cognitive development in DS. Two important DMRs, overlapping promoters of CPT1B and cardiomyopathy associated 5 (CMYA5), were recently associated with hippocampal volume in non-DS individuals; DNA methylation changes in DS newborns were associated with smaller hippocampal volume.[171] Additional DS-associated DMRs overlapped NudE neurodevelopment protein 1 (NDE1), PR/SET domain 8 (PRDM8), and the enhancer locus for Hes family BHLH transcription factor 1 (HES1), genes that also promote neurogenesis.[172–174]

The most significant DMR outside of Hsa21 is FLI1 (Fli-1 proto-oncogene, ETS transcription factor), an important regulator of megakaryopoiesis.[175,176] It is located at the promoter of transcript variant 4 that is widely expressed in megakaryocytes. FLI1 binds both RUNX1 and GATA1 during terminal megakaryocyte maturation and all three proteins cooperate in transcriptional control of megakaryocyte differentiation.[177,178] Similar to RUNX1, germline loss of FLI1 has been associated with thrombocytopenia, defects in megakaryopoiesis, and familial platelet disorders.[176,179,180] FLI1 expression is significantly reduced in DS FL myeloid progenitor cells.[18] T21 leads to epigenetic dysregulation of both RUNX1 and FLI1, which may contribute toward abnormal megakaryocyte development in DS FL cells, and to the development of TAM and the concomitant risk of AMKL in DS infants.[18,181] Along with RUNX1, FLI1 is also a critical regulator of embryonic hematopoiesis; thus, compensatory epigenetic downregulation of RUNX1 and FLI1 may be required for viable embryogenic development in DS, but potentially also results in increased risk of hematological malignancies.[159,182]

GATA1-truncating mutations confer a growth advantage to fetal hematopoietic cells and are clonally selected during development of TAM. The term GATA is derived from zinc finger proteins that bind the consensus DNA sequence (T/A)GATA(A/G). GATA1 mutations in DS are related to Ts21-associated upregulation of GATA1; increased transcription can promote DNA mutagenesis.[181,183] Epigenome-wide association studies (EWAS) of GATA1 mutations in DS revealed a DMR overlapping Vault RNA 2-1 (VTRNA2-1), a metastable epiallele at which DNA methylation levels were previously associated with the periconceptional environment, suggesting a potential environmental role in the development of GATA1 mutations.[18,184] All genome-wide studies have shown that Tsa21 produces significant changes in the expression of genes on the other (non-trisomic) chromosomes, indicating perturbations of downstream transcriptional networks.[20]

The N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) and DNA methyltransferase 3 like (DNMT3L) genes encode methyltransferases for cytosines in DNA. DNMT3L heterodimerizes with DNMT3A and DNMT3B to form catalytically active DNA methyltransferases or with other cellular components that may compete for methyl groups from S-adenosyl methionine (SAM; N6AMT1). Lastly, the protein coded by the Hsa21 MIS18 Kinetochore Protein A (MIS18A) gene interacts with DNMT3A/3B and has been shown to be critical for maintaining DNA methylation at centromeres.[185] The increased dosage of these genes could in principle affect DNA methylation in cells with Ts21. Certainly, increased expression of DNMT3L must be considered as a possible

explanation for the predominance of hyper-methylated DS-DM loci in placenta and fetal and adult cerebrum.

Carnitine Palmitoyltransferase 1B showed increased gene body methylation and mRNA expression in DS beginning in early gestation.[135] Differential CPT1B methylation was also reported in blood leukocytes, buccal epithelial cells, placenta, and brain of DS patients.[22,65,67,69] Carnitine Palmitoyltransferase 1B on chromosome 22 is one of 3 carnitine palmitoyltransferase 1 genes, expressed in heart and skeletal muscle.[186]

Epigenetic Mechanisms of DS-DM

Two different scenarios for trans-acting molecular mechanisms, one related to the high dosage of Hsa21-linked SAM-dependent methylation pathway genes, and second, increased Hsa21-linked TF genes, have been postulated to underlie DS-DM.

In the "methylation pathway hypothesis", Hsa21 contains many genes with known/predicted roles in DNA methylation.[13,20,63,187] The Solute Carrier Family 19 Member 1 (SCL19A1) gene codes for the reduced folate carrier protein that acts as a transporter at the plasma membrane, while the products of the superoxide dismutase 1 (SOD1), glycinamide ribonucleotide formyltransferase (GART) and cystathionine beta-synthase (CBS) genes act in metabolic pathways involving folic acid and homocysteine in the cytoplasm. Pogribna et al. have presented evidence that the decreased availability of homocysteine caused by CBS over-expression in proliferating cells with Tsa21 promotes a functional folate deficiency – which could account for a mild but significant global DNA hypo-methylation in blood cells from individuals with DS.[63] Unlike in the brain and placenta, most DS-DM loci in blood cells show hypo-methylation and a role of folate deficiency in producing DS-DM in those tissues seems doubtful.

The N6AMT1 and DNMT3L genes encode enzymes or components of enzymes that act as methyltransferases, either for cytosines in DNA (DNMT3L, which codes for a protein that heterodimerizes with DNMT3A and DNMT3B to form catalytically active DNA methyltransferases) or for other cellular components that could compete for methyl groups from SAM/N6AMT1. Lastly, the protein coded by the MIS18 Kinetochore Protein Homolog A (MIS18A) gene in infants with Hsa21 interacts with DNMT3A/3B and has been shown to be critical for maintaining DNA methylation at centromeres.[183] The increased dosage of these genes could in principle affect DNA methylation in cells with Tsa21. Certainly, increased expression of DNMT3L must be considered as a possible explanation for the predominance of hyper-methylated DS-DM loci in placenta and fetal and adult cerebrum.

Early Epigenetic Aging in DS

Amyloid precursor protein triplication is required for early onset Alzheimer-like aging in DS.[188,189] These changes could be a mimic or "phenocopy" of early aging, not actually due to it. Alternatively, increased APP gene dosage might promote chronological aging.[190,191] According to this hypothesis, the age-at-onset of AD in DS is dictated by multiple modifier genes, including some that are not located on Hsa21 but have biological roles in AD pathology and are genetically polymorphic in human populations, and others, aside from APP, that reside on Hsa21 and might affect the aging process.[192,193]

Epigenetic Clock

Horvath devised a new molecular marker of aging, referred as "epigenetic clock" based on changing DNA methylation at different chronological ages in humans and mice.[194,195] A weighted average of fractional methylation levels of a set of 353 "clock CpGs" was noted and then transformed to DNA methylation age of biological samples using a calibration function. Differences between methylation and chronological age can be defined as the residual that results from regressing DNA methylation age on chronological age. In non-DS individuals, the DNA methylation age is strongly correlated with the chronological age in multiple cell types and tissues, including neurons and glial cells.[194]

To determine whether the "epigenetic clock" confirmed the accelerated aging due to Ts21, DNA methylation datasets were analyzed, including blood samples and various brain areas.[135] A strong correlation was noted between the chronological age and DNA methylation in non-DS controls, which helped establish a reference regression line. The predicted (estimated) age in controls is referred to as DNAm age; it is highly correlated with chronological age in CD4 T-cells, monocytes, and neurons.[194,196] Down syndrome sample showed an accelerated aging effect (on average by 6.6 years), particularly in the frontal lobe and cerebellum.[135] Alzheimer-like neuropathology could be seen as early as 40 years of age, earlier than in the general population.[12] The epigenetic clock is a useful quantitative tool; it is an improvement over the telomere length measurements and is applicable in all types/sources of cells/tissues, including cryopreserved banked specimens.[194]

Down syndrome cells show a faster aging pattern of CpG methylation patterns during the fetal/neonatal period and early childhood, which has helped establish an initial "set point" of epigenetic age.[20] More data are needed.[65] Interestingly, the changes of DS-related accelerated aging can be segmental, where the changes are more prominent in some areas but not in others, and in brain than in blood.

Animal Models of DS

Canzonetta et al. reported a 30–60% reduced expression of neuron-restrictive silencer factor (NRSF) and increased downstream genes in the trans-chromosomic TgDyrk1A mouse model of DS.[24] This inverse correlation was not seen in another transgenic mouse model of DS that overexpressed DYRK1A.[43] However, silencing the third copy of DYRK1A by RNA interference rescued NRSF levels, confirming the role of DYRK1A in NRSF-mediated gene regulation.[24] DYRK1A regulates NRSF by binding to the SWI/SNF chromatin remodeling complex.[43] This complex uses ATP to mobilize nucleosomes and rearrange the chromatin structure and induces the expression of multiple other genes involved in histone modifications, for example, the histone methyl transferase L3MBTL2, the histone demethylase Jumonji/ARID domain-containing protein 1D (JARID1D), and the HDAC interactor, the nuclear receptor corepressor (NCoR).[43,46,197] Therefore, DYRK1A overexpression in DS is likely to affect a range of epigenetic mechanisms.

The contribution of DYRK1A to learning and memory deficits in DS is further supported by findings from Altafaj et al.[198] They administered short hairpin RNA against DYRK1A to Ts65Dn mice and showed normalized DYRK1A protein levels, improved synaptic plasticity, and partial amelioration of the hippocampus-dependent visuospatial memory (VSSM; Morris water maze). Another study showed that the DYRK1A inhibitor epigallocatechin-gallate (EGCG) rescued VSSM and object recognition memory (novel object recognition test) in both Ts65Dn and TgDyrk1A mice. However, EGCG-treatment in a pilot human study (3 months) in young adults with DS did not show consistent improvement.[199]

In addition to DYRK1A, two other HSA21-encoded proteins interact with the SWI/SNF complex, thereby altering histone modifications and gene expression: BRWD1 and RUNX1. The bromodomain and WD repeat-containing 1 (BRWD1) modulate the chromatin by binding through its two bromodomains and by associating with the SWI/SNF complex.[44] Runt-related transcription factor 1 forms multiprotein complexes at target gene promoters to which the SWI/SNF subunits BRG1 and INI1 bind. Runt-related transcription factor 1 is associated with histone modifications that are typical of euchromatin, such as dimethylated H3K4 and acetylated H4.[45] Runt-related transcription factor 1 gene was found to be hypermethylated in DS compared with controls, suggesting altered RUNX1 gene expression.[64,79] Consequently, altered RUNX1 protein levels likely affect epigenetic marks as well.

Increasing evidence suggests the involvement of SWI/SNF complex chromatin–remodeling complex in neurodevelopment and hence might be critical in the cognitive deficits in DS. The expression of the SWI/SNF subunit BRG1 is enriched in the brain and the spinal cord of mice embryos and the dorsal neural tube of chick embryos.[200,201] The alpha-thalassemia X-linked intellectual disability (ATRX) syndrome is caused by mutations in the gene that encodes the SWI/SNF protein ATRX.[202] Therefore, aberrant functioning of the SWI/SNF complex due to overexpressed HSA21 products might lead to intellectual disability in a similar way.

Epigenetic Therapy May Alleviate Cognitive Deficits in DS

As mentioned above, DS mouse models (Ts65Dn mice) can be useful for studying cognitive deterioration in DS.[203] Drugs that inhibit epigenetic enzymes, known as epidrugs, can be tested in these models.[61] One, the DYRK1A inhibitor EGCG[204] can promote the re-expression of genes that were silenced through promoter methylation.[205,206] Ramakrishna et al. reported low expression of the presynaptic α-synuclein protein in brain tissue in DS individuals and Ts65Dn mice.[207] Epigallocatechin-gallate treatment of Ts65Dn mice resulted in increased DNA methylation of the increased DNA methylation of the alpha-synuclein promotor, and increased expression of the alpha-synuclein protein.[208] Further study is needed in DS individuals.

Epidrugs carry a risk of considerable adverse effects because of genome-wide and nonchromatin effects. In combating the cognitive deficits in DS, specific targeting of the third copy of Hsa21 in the brain is required without affecting peripheral epigenetic modifications. The complete third copy can be silenced *in vitro* using the large non-coding RNA molecule X-inactive specific transcript (XIST). Jiang et al. introduced an inducible XIST transgene into the DYRK1A locus in induced DS pluripotent stem cells. Stable chromosome-wide transcriptional silencing with heterochromatin marks were observed. The transcription of DYRK1A and APP was repressed.[209]

Epigenetic editing offers a targeted approach to modulate the expression of individual genes, such as DYRK1A and APP in DS models. These strategies target specific epigenetic enzymes (writers or erasers) to specific genes with lab-engineered DNA-binding domains.[210] Engineered domains are fused in desired epigenetic enzymes, such as specific DNMTs, DNA demethylases, or histone modifiers.[211–218] These changes can lead to long-term modulation of gene expression.[210,211]

Epigenetics in DS: A Link to Alzheimer's Disease?

Postmortem analysis revealed that virtually all DS individuals have an extensive Alzheimer's disease-like neuropathology from 40 years of age, though not all are symptomatic.[12] The triplication of the APP gene has been regarded as the main cause of the heightened risk of cognitive deficits in DS, but epigenetic mechanisms are known to affect gene expression and are thus potential therapeutic targets to interfere with Alzheimer disease-like neuropathology in DS.[219,220] Besides phosphorylating APP at threonine residue 668,[221] DYRK1A can phosphorylate multiple sites of tau proteins and contribute to these pathological changes.[222] MicroRNAs can also play a role by promoting the condensation of heterochromatin.[35,223,224] MicroRNA-125b is located on Hsa21 and thus overexpressed in DS.[225]

Future Therapies

Methyl donor supplementation with folic acid, betaine, and vitamin B_{12} has shown promise in DS individuals.[187] Similarly, *in vitro* studies have shown that altered balance of metabolites in SAM-dependent methylation pathways in Tsa21 cells can be altered by adding methionine, folinic acid, methyl-B12, thymidine, or dimethylglycine.[63] Methyl donor-rich diets may not consistently alter the hypermethylation but might prevent hypomethylation in DS-DM loci in brain cells. The identification of specific DS-DM target genes that play roles in DS-associated phenotypes such as immune dysregulation and intellectual disability may be avenues to future-targeted therapies.

Conclusion

Many overexpressed products of Hsa21 genes are epigenetic modulators and can explain the observed learning and memory deficits. Epigenetic marks are reversible; epigenetic editing, where specific epigenetic enzymes are recruited to specific genes by using laboratory-engineered DNA binding domains can help in consistent modulation of gene expression. Partial repression of overexpressed Hsa21 genes can possibly alleviate some the cognitive deficits in DS. Further studies are needed.

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REVIEW ARTICLE

Fetuses can Listen, Learn, and Remember: We Need to be Cautious about What and How We Say It!

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Abstract

The fetal auditory system becomes functional during mid-gestation or possibly even earlier. Existing data show that fetuses can respond to maternal voice and different types of music, both vocal and instrumental. The ability to receive and transmit sound waves, and then recognize and retain some memory of these auditory stimuli could possibly be one of the most important developmental sensory milestones that we need to learn about. Unfortunately, we still have limited evidence for the precise role and timing of prenatal sound simulation. There is a need for methodologically strong, randomized controlled trials with rigorously designed interventions and standardized reporting measures. We may need to compare different durations and types of musical (sound) intervention. At a minimum, these interventions can improve maternal–fetal bonding and family-centered outcomes. Any evidence of neurodevelopmental gains would be an important scientific/medical advancement. In certain conditions such as neonatal abstinence syndrome, emerging evidence suggests that early, *in utero* intervention with music therapy can be helpful; these findings bring hope for new therapeutic tools to enhance the neurological development of at-risk fetuses. Considering that prenatal music exposure might have positive effects on the fetus and newborn infant, we need carefully conducted studies of intrauterine neurosensory organization with long-term follow-up.

Keywords: Fetus, Mother's cardiotocographic parameters, Music therapy, Neonatal behavior, Neonatal neurological system, Newborn, Pregnancy, Rhythm, Sound, Speech.

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Key Points

- In terms of structure, the fetal hearing system is recognizable at early as 3–6 weeks of pregnancy. Functionally, the inner, middle, and outer ear are sufficiently developed at 24–25 weeks' gestation to identify/distinguish between vibroacoustic stimuli.
- Fetuses and newborn infants can respond to maternal voice across her abdominal wall. They also respond to different types of music, both vocal and instrumental.
- *In utero* exposure to music and speech induces stimulus-specific memory traces in the fetus. These stimuli may promote brain growth and possibly even short- and long-term cognitive gains.
- There is some evidence that music therapy can help in disorders such as neonatal abstinence syndrome. Possibly, timely *in utero* intervention may also enhance neurological development in other high-risk conditions.
- We need to carefully evaluate the immediate and long-term effects of music therapy on intrauterine neurosensory organization.

Introduction

The fetus is exposed to a variety of auditory stimuli from as maternal heart sounds, voice, respiratory and bowel sounds, and environmental stimuli.[1] Since hearing is acquired early during pregnancy, there is a possibility that planned antenatal exposure to music might have a have a role in fetal learning.[2,3] Considering that music also has a calming effect on the mother with few known adverse effects,[4] There is curiosity as to whether music could/should be a routine intervention during pregnancy.[5] In this article, we have reviewed the information available on the impact of prenatal exposure to music on the growing fetus. We have assimilated

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information from an extensive review of the literature utilizing key terms in multiple databases including PubMed, EMBASE, and Science Direct.[6,7] To avoid bias in the identification of studies, keywords were short-listed *a priori* from anecdotal experience and PubMed's Medical Subject Heading (MeSH) thesaurus.[8–12]

The Beginnings of Sound Reception in Fetal Development

At the time of birth, a newborn baby is largely equipped with all the senses available to an adult.[13] The ability to receive and transmit stimuli in sound waves is important and matures rapidly in the second trimester.[14] Training becomes possible in later pregnancy.[15]

The development of the auditory system begins between 3 and 6 weeks of pregnancy.[16] At 24–25 weeks' gestation, the structures of the inner, middle, and outer ear are sufficiently developed to recognize vibroacoustic stimuli.[17] In the developing central nervous system (CNS), auditory stimuli can promote the maturation of the temporal lobe regions that help recognize low-frequency sounds.[18] The fetus hears sounds coming from the mother's body first, such as those emanating from her cardiac contractions, blood flow in larger vessels, intestinal peristalsis, or airflow in the respiratory tract. The hearing threshold between 27 and 28 weeks' gestation is about 40 dB.[19,20] The fetuses then respond to sounds with a frequency of 250–500 Hz.[21,22] Ongoing myelination of nerve fibers in the CNS continues to improve impulse conduction.[23] Between 29 and 30 weeks' gestation, the fetus can perceive higher-frequency sounds such as the mother's voice, an infant's cry, or an alarm.[24] During the 34–35 weeks period, fetuses no longer seem to get alarmed with sounds that previously caused arousal. They begin to respond to sounds with frequencies of 1000–3000 Hz. At term, the hearing threshold is 13.5 Hz.[25]

During pregnancy, extraneous sounds are usually first transmitted from the air, through the abdominal wall, the uterus, and finally to the amniotic fluid and the head of the fetus.[26] Intra-uterine sounds might be more important. The sound of the mother's heartbeat is 25 dB above basic noise, dominating the fetal environment.[24] Her voice is heard almost 4 times louder in the womb than outside.[27] Most sounds in the fetal environment are dominated by lower frequencies.[28] Energy above 0.5 kHz is attenuated by 40–50 dB. The fetus easily detects vowels, whereas consonants, which are higher in frequency and less intense than vowels, are largely unavailable.[28]

The Importance of Music in the Prenatal Period

Music stimulation of a growing fetus is plausibly a positive stimulus to promote a range of positive outcomes for both the mother and the fetus, the child.

Emotional Bonding and Stress Reduction

Long-standing cultural practices view music as a likely catalyst for emotional connection, allowing a mother to establish a deeper bond with her unborn child.[29] Soothing songs and singing lullabies have been encouraged as a means to decrease stress and promote relaxation and emotional well-being. The harmonious vibrations of music can help to create a serene environment, fostering a sense of peace and tranquility for both the mother and baby.

Language Development and Cognitive Stimulation

Prenatal music and speech form stimulus-specific memory traces during the fetal period,[30,31] thereby stimulating brain growth, cognitive development, and long-term neural effects.[32] Fetuses start responding to music in the second trimester.[33] Singing by pregnant women provides auditory stimulation to their unborn child.[33,34]

Language acquisition and development of hearing could be enhanced by rhythmic melodies.[33,34] Cognitive abilities and habituation for music can be demonstrated in fetuses and young infants.[35] Arguably, the ability to discriminate speech stimuli *in utero* can promote acquisition of native language fundamentals during fetal life.[36] Neonates prefer their mother's voice and also the language their mothers used while pregnant with them.[37–39]

Sleep Regulation and Relaxation

In newborn infants, soothing melodies and instrumental compositions can help establish healthy sleep patterns in both the mother and a newborn infant.[40] Similarly, music can promote natural sleep in both the pregnant mother and her fetus by reducing stress.[41] As shown in late preterm infants, these effects could possibly be mediated by the release of endorphins in the CNS.[40,42,43]

Bonding and Maternal Heartbeat

In the antenatal period, mother's heartbeat is a familiar and comforting rhythm for the baby.[44] Rhythmic melodies and instrumental compositions can simulate maternal heartbeat and promote mother–fetal bonding, which might extend into the postnatal period.[29,45–47]

Enhanced Sensory Development

Music can promote the development of the fetal sensory pathways and sensory integration.[31] Vibrations caused by music can be felt by the fetus, creating a multisensory experience that involves hearing, movement, and consequently, touch with the uterine walls, the umbilical cord, and even its own face.[42,48,49] The movements indicate their engagement with the auditory stimuli, thereby preparing them for the postnatal sensory experiences.[30]

Maternal–Fetal Interactions

Fetal activities can be observed and followed since early pregnancy.[50] The maturation of these activities is closely related to the development of the sense organs even *in utero*. Fetal motor activity can be identified as early as at 6–8 weeks' gestation.[51] At 12 weeks, the movements can now be characterized as "kicking," "fetal rotation," "opening/closing of the fist," "nodding," "sucking-like oral activity," and "reactions to touch."[51] As pregnancy progresses, these activities become more frequent, mature with larger amplitude and improved coordination of muscle groups.[52] These movements are brief but the frequency progressively increases toward term gestation.[53]

In 2015, Marx and Nagy showed that fetuses reacted with arm, head, and oral movements when the mother touched the belly.[54,55] Her voice evoked head and arm movements. Third-trimester fetuses showed more yawning (regulatory), resting (crossed arms), and independent touch (hands touching body) responses to stimuli compared with those seen during the second trimester. Two years later, the same investigators used 3- and 4-dimensional ultrasound to examine fetal responses to tactile stimuli on maternal abdomen. Touching the belly by the mother, father, and a stranger was compared. In the control group, the fetal response was examined while at rest, without touching the abdomen. When the mother touched her abdominal wall, the third-trimester fetus touched the uterine wall for significantly longer periods than those in the second trimester. The third-trimester fetuses showed stronger/longer responses when touched by their mother. These differential

responses in older fetuses may reflect the maturation of the CNS and the emergence of proprioceptive self-awareness.[54]

In 2021, Nagy at al.[56] examined fetal responses to maternal touch and voice. The sensorimotor competencies of the fetus clearly suggest that communication readiness begins to develop prior to birth. In response to maternal abdominal touch, fetuses show responses that have been variably perceived by different observers as cross-communication or self-stimulation. When the mother touched her abdominal wall and spoke in a gentle voice, fetal movements paused for some time. Similar responses were seen during conversations with a third person (interpersonal conversation). The fetuses showed a longer mouth-opening time during these period. The results suggest that third trimester fetuses can distinguish between interactive and non-interactive external stimuli and also distinguish these from incidental, other interactions.

In 2012, Kisilevsky et al.[57] examined the impact of auditory stimuli on fetal heart rate (HR) and heart movements in diabetic and overweight pregnancies. Fetuses in overweight pregnancies responded to their mother's voice with increased HR. These altered reactions to the maternal voice in diabetic pregnancies may indicate relatively immature nervous or auditory system, increased sensorineural threshold, lower levels of thyroid hormones, or iron deficiency in the mother.

Lee et al.[58] compared fetuses in women with hypertension vs controls upon hearing maternal voice. In the maternal hypertension group, the fetuses showed increased HR upon listening to the mother's voice. However, if the voice was recreated, there was no increase in fetal HR. The differential responses of the fetus to the maternal voice in pregnancies complicated by maternal hypertension may reflect a functional increase in the sensorineural threshold or a delay in the maturation of the auditory system, which may indicate functional differences during fetal life or subtle differences in the development of the CNS. Recent observations of maternal voice recognition provide evidence for residual memory and learning in healthy fetuses. We still need to investigate higher-order auditory processing in maternal hypertension, which is associated with reduced and/or compromised uteroplacental blood flow.

Knowledge of memory is based largely on observing newborns who seem to recognize their mother's voice. A few studies have assessed the biological processes that facilitate fetal responses to the maternal voice *in utero*.[59] Fetal HR and motor activity have been recorded at 36 weeks' gestation while pregnant women read aloud from a neutral passage.[60] Compared with the resting period, fetuses showed reduced motor activity and slower HR within 10 seconds after the onset of maternal speech. Subsequent analyses showed that the fetal response was modified by both maternal and fetal factors. The behavior of fetuses whose mothers were previously awake and talking was analyzed; a period of fetal orientation can be measured when a mother starts reading aloud. Compared with women who rested and remained silent, the fetuses reacted with increased HR and movements. The fetal response was also dependent on the initial variability of the fetal HR; the greatest response was shown during periods of low variability, when the mothers had been resting and were silent. The results indicate that fetal response is influenced by both maternal and fetal conditions, which has an impact on understanding the fetus's learning of the mother's voice in neutral settings.[60] The reaction to sounds was also compared with those of premature infants. In high-risk infants, the cardiac and motor responses were recognizable at 30 weeks' gestation. At 33 weeks, high-risk infants showed increased HR compared with low-risk fetuses. The results indicate that both

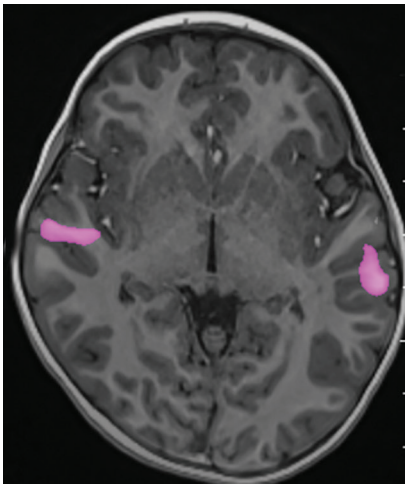

Fig. 1: Sounds activate specific temporal lobe regions in children: A passive-listening functional-magnetic resonance imaging (fMRI) task comprised of a series of words and non-language noise induced bilateral activation in the posterior superior temporal gyri. This particular fMRI image is from a 5-year old with a hypothalamic hamartoma who underwent fMRI for surgical planning; sounds were delivered using headphones. The image shows that we now have the technology to study the impact of sounds on specific areas of the brain and can study developmental changes in young infants

low- and high-risk premature infants begin to respond to sounds at the same gestational age. The varied responses observed during pregnancy in the high-risk group most likely indicate diversified functional development of the auditory system.[61]

The maturation of the fetal response to music was characterized in the last trimester of pregnancy using a 5-minute piano recording of Brahms' Lullaby played at an average volume level of 95, 100, 105, or 110 dB.[62] Within 30 seconds of turning on the music, the youngest fetuses (28–32 weeks of gestation) showed an increase in HR limited to the two highest dB levels. During pregnancy, the threshold level decreased and a shift in response from acceleration to deceleration was observed at lower dB levels, indicating attention to the stimulus. During 5 minutes of music, fetuses over 33 weeks of gestation showed a sustained increase in HR. Changes in body movement occurred at 35 weeks of pregnancy. These findings suggest a change in the processing of complex sounds around the 33rd week of gestation, with response limited to the acoustic properties of the signal in younger fetuses, while episodes of focusing were observed in older fetuses.[62]

An attempt was also made to examine the impact of lullabies sung by mothers on the bond and behavior of newborns. There was no significant effect on prenatal attachment. Only the postnatal bond was significantly greater in the group of singing mothers 3 months after birth. In the same group of singing mothers, episodes of newborn crying in the first month, as well as infantile colic, were significantly less common. At the same time, a reduction in the frequency of night awakenings was observed. It can be concluded that singing lullabies by mothers can improve the bond between mother and child. It may also have a positive impact on the behavior of newborns and thus reduce the stress level in the young mother (Fig. 1).[63]

The Mozart Effect

There are two complementary communication systems in humans—language and music and functional imaging studies suggest these

are processed in nearby brain regions. Music affects many aspects of human behavior, encourages social interactions and promotes trust and cooperation within groups of culturally compatible individuals. Music acts on the limbic system, is rewarding and motivating, and can facilitate learning and memory.[64–66]

Oxytocin is vital to the attachment between infants and parents through early contact and interaction can influence developing brain functions in infants culminating in behavioral changes in the child.[67,68] Maternal behavior is mediated by oxytocin, a neuropeptide synthesized in the paraventricular nucleus (PVN).[69–71] The central nucleus of amygdala (CeA) expresses oxytocin receptors and interacts with the reward circuit to motivate maternal behaviors.[72–74] Experiments in rats have shown that maternal behaviors are influenced by environmental factors in pregnancy; being reduced by stress and enhanced by enriched environments. The "Mozart effect" influenced the licking of offspring by mother rats and such offsprings had decreased behavioral anxiety and stress responses as adults.[75,76] Increased maternal oxytocin levels lead to greater affectionate contact behaviors in mothers following mother–infant contact.[67] The changes in rearing behaviors due to music are due to modulation of the oxytocinergic system by activation of oxytocin receptors and neuromodulation of the oxytocinergic system.[72,74,77]

Placental Programming

Hereditary and environmental stimuli are assimilated as placental epigenetics which influence the fetal development. Fetal brain development in the third trimester is impacted by plasticity and hence neurobehavioral phenotype can be altered by prematurity. Prenatal music stimulation has a positive epigenetic effect on fetus by virtue of placental programming.[78]

The auditory cortex is located in the posterior-medial part of Heschl's gyrus. This region corresponds with the Brodmann's area 41, a highly plastic epigenetic area vital to prenatal learning.[79,80] Acoustic environments influence the functional organization and processing capabilities of the auditory cortex.[81,82]

Music Therapy and Memory

Musical intervention has a therapeutic role in dementia or Alzheimer's disease because music elicits feelings and memories.[83] Music therapy induces plastic changes in brain networks, thereby facilitating brain recovery processes and modulation of emotions and communication.[84] Hence, it is a propitious modality of rehabilitation.

It has been reported that listening to classical music, specifically selections from Mozart can result in a temporary improvement in cognitive functions like abstract/spatial reasoning tests.[85] The "Mozart Effect" is attributed to the arousal due to the pleasure of listening to music, rather than a direct impact on cognitive ability.[86,87]

Influence of Music on Fetal Cardiotocographic Parameters

Although music has long been recognized for its effect on human emotions, physiological responses, and overall well-being, the mechanisms remain largely unknown.[88] Machin and Dunbar proposed that activation of the endogenous opioid system, including β-endorphins and encephalins, which are known to foster and maintain social bonds, improve mood and reduce the sensation of pain, are a possible mechanism.[89] In women with a low- or high-risk pregnancy, and in non-stress tests (NSTs), music appeared to reduce maternal anxiety.[90–92]

During pregnancy, maternal stress can affect fetal development, including altered fetal HR patterns.[93] In a recent systematic review, Shimada et al.[94] noted improved maternal relaxation, decreased anxiety, psychosocial stress and depression, reduced pain, increased maternal bonding with her fetus, improved sleep quality, improved blood pressure profiles, lower fetal HR, and lower need for medications after surgery. Music therapy during the pre- and perinatal periods may be benefit both the pregnant women and their newborns infants. Maul et al.[95] performed a systematic review to assess maternal mental health outcomes after musical intervention from 14 randomized controlled trials (RCTs) including 2,375 pregnancies. They showed reduced maternal stress, anxiety, and depression. These data are consistent with the beliefs that modulation of maternal physiological parameters through music may influence fetal well-being.[5]

Neural processing of music involves an extremely complex and extensive network of cortical and subcortical structures which integrate auditory, sensory motor, and cognitive functions as well as emotional changes.[4] As expected from our understanding of the neurosensory development of the fetal auditory system, initial responsiveness to different sound frequencies begins around 23 weeks' gestation. By 24 weeks, fetuses demonstrate a startle response to vibroacoustic stimulation. These responses are seen consistently at 28–30 weeks.[33,96,97] Indeed, music may improve fetal autonomic responses and learning/memory formation.[30,32,98]

Evidence from RCTs

Effect of Music Exposure on FHR

In a prospective RCT, James et al.[99] examined whether prenatal exposure to a musical stimulus altered fetal behavior and whether these responses persisted after birth into the newborn period. Using an exposure-learning model, music was played to 10 fetuses via a headphone on the maternal abdomen and 10 controls had the headphone without sound. All fetal studies took place within 72 hour prior to elective delivery. After delivery, all 20 newborns were exposed to the same music on postnatal days 3–5. Computerized assessment of FHR and activity was documented and neonatal behavioral states were recorded. For the 1st hour of the study, neonates that had had *in utero* exposure showed higher mean HR and showed longer periods of high HR variation. These findings, however, were not statistically different from controls. However, by the 4th hour, the exposed fetuses began to show significantly more HR variation ($p = 0.04$) and more state transitions ($p = 0.01$) compared with unexposed fetuses. These effects persisted into the neonatal period with the same music stimulus evoking more state transitions ($p = 0.01$) and leading to longer awake states ($p = 0.05$). Thus, prenatal exposure to music altered the fetal behavior that persisted after birth.

Granier-Deferre et al.[100] showed that repetitive prenatal exposure to specific melodies influenced neonatal auditory perception and memory that was retained for 3–4 days to 6 weeks. In the test arm of the study, fetuses were given precisely controlled exposure to a descending piano melody twice a day during weeks 35–37 of gestation. After 6 weeks, cardiac responses of 25 exposed infants and 25 naive control infants to the descending melody and to an ascending control piano melody were examined during quiet sleep. The melodies had precisely inverse contours, but similar spectra, identical duration, tempo and rhythm, and thus, nearly identical

amplitude envelopes. All infants displayed a significant change in HR. In exposed infants, the descending melody evoked a cardiac deceleration that was twice larger than the decelerations elicited by the ascending melody and by both melodies in control infants.

Brillo et al.[101] randomized 30 healthy mother–fetus dyads in a 1:1:1 ratio to one of three groups: (1) fetuses were submitted to pre-listening phase (33^0–36^3 week) and listening sessions during 4 NST; (2) fetuses were submitted to listening sessions during 4 NST only; and (3) 4 NST without any listening. Mean fetal HR, fetal HR accelerations/decelerations, fetal movements, and uterine contractility were assessed. The 1st group fetuses, who had heard a particular piece of music during previous sessions, showed significantly increased HR accelerations and movements during the music listening session of the last NST. Uterine contractions did not change in frequency. They concluded that fetuses respond to familiar but not to unknown music.

Catalgol and Ceber Turfan randomized 100 (50 intervention, 50 control) primipara women. The NST was applied in 36–38 weeks' gestation.[102] During the test, music was played to the intervention group, while the control group received routine care. The music group showed lower mean scores of State Anxiety Inventory during NST. Acceleration, mean number of fetal movements and fetal HR reactivity were significantly higher in the intervention group. Thus, music therapy in pregnant women decreased maternal anxiety and had positive effects on NST findings.

Soylu et al.[103] showed that music affected vital signs, fetal movements, and lowered the state and trait anxiety levels during NST in pregnant women. In 74 (37 music and 37 control group) pregnant women, post-music exposure HRs were lower than the pre-procedure values ($p < 0.001$). The groups did not differ in baseline fetal HR, variability, fetal movement, presence/number of accelerations-decelerations, and NST parameters. The number of fetal movements was higher in the music group than controls ($p < 0.001$). The state anxiety inventory scores were lower in the music group than controls ($p < 0.001$).

In a cross-over RCT, Oh et al.[104] tested the effects of musical intervention on maternal anxiety, fetal HR, and testing time during NST. Sixty pregnant women in 28–40 weeks' gestation were randomly assigned to either an experimental ($n = 30$) or a control group ($n = 30$). The experimental group showed significantly lower scores in state anxiety than controls. There two groups showed no difference in systolic blood pressure and HR. Baseline fetal HR was significantly lower and frequency of acceleration was significantly increased in the experimental than in the control group.

Estrella-Juarez enrolled 343 full-term pregnant women in a RCT and divided them into three parallel groups: (1) music therapy intervention ($n = 104$), (2) virtual reality intervention ($n = 124$), and (3) controls ($n = 115$).[105] The interventions were delivered during NST in the third trimester and during labor. Measures included the Spielberger State-Trait Anxiety Inventory, maternal blood pressure, maternal and fetal HRs, and labor and birth outcomes. Women in the music therapy and virtual reality groups had less anxiety after NST ($p < 0.001$), and the women were more likely to have a reactive NST ($p < 0.001$) than controls. Following completion of NST and intervention, music therapy and virtual reality groups had lower systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), and maternal HR ($p = 0.003$) than controls. Furthermore, fetuses in the control group were more likely to show non-reassuring fetal HR tracings than those in the music therapy and virtual reality groups, respectively ($p = 0.004$).

Effect of Prenatal Music Exposure on Neonatal ECG and Neuro-behavioral Response

In a pilot study, Lang et al. showed that newborns displayed distinct reactions to maternal voice at 2 and 5 weeks after birth on a physiological level and identifiable with ECG and EEG changes.[106] Basic memory traces were formed *in utero* and shaped neonatal autonomic and neuronal reactions to speech and voice stimuli. Newborns exposed to nursery rhymes prenatally showed distinctly different reactions than those not exposed. The authors concluded that fetal brain is "programmed" for the predicted postnatal environment and maternal voice.

In an open-labeled RCT, Arya et al.[3] evaluated the effects of antenatal music exposure in healthy primigravidae on the behavior of their term appropriate-for-date newborns, assessed using 7 clusters of the Brazelton Neonatal Behavioral Assessment Scale (BNBAS). Primigravida mothers aged 19–29 years who had a singleton pregnancy, at ≤20 weeks' gestation, and did not have any chronic medical diseases or significant hearing impairment, were randomized to listen to a pre-recorded music cassette for approximately 1 hour/day in addition to standard antenatal care (intervention arm) or standard care only (control arm). Perinatal factors with adverse effect on neonatal behavior were deemed as protocol violations. One hundred and twenty-six newborns (music group) and 134 (controls) were tested. Infants of mothers exposed to music during pregnancy performed significantly better on 5 of the 7 BNBAS clusters. The maximal beneficial effect was seen with respect to orientation (ES 1.13, 95% CI: 0.82–1.44, $p < 0.0001$) and habituation (ES 1.05, 95% CI: 0.53–1.57, $p = 0.0001$). Prenatal music exposure to mother significantly and favorably influenced neonatal behavior.

As seen above, many systematic reviews were of mixed methodological quality and showed ambiguous efficacy of auditory stimulation of preterm infants. Hence, a meta-analysis of several studies was performed.[107–132] The authors evaluated the impact of parallel and cluster-RCTs on preterm infants <37 weeks' gestation during hospitalization, and also studied the effects on parents who were involved in the intervention. The evaluated interventions included any live/recorded music or vocal stimulation for >5 minutes, administered >3 times, by a music therapist, a parent, or a healthcare professional, and these were compared with standard care. Many studies had to be excluded from this analysis because of inadequate data.[42,133–191]

The authors studied 25 trials including 1,532 infants born at 25–36 weeks' gestation and 691 parents (21 parallel-group RCTs, 4 cross-over RCTs). The intervention did vary in type, delivery, frequency, and duration; music and voice that were typified as calm, soft, musical parameters in lullaby style were often integrated with mother's voice. There was considerable variability in the risk of bias in included studies. Music/vocal interventions reduced HRs in infants during intervention (mean difference, MD, −1.38, 95% CI: −2.63 to −0.12; $p = 0.03$; 1014 infants; 11 studies; moderate-certainty evidence) and after intervention (MD −3.8, 95% CI: −5.05 to −2.55; $p < 0.00001$; 903 infants, 9 studies; high-certainty evidence). There were no reported adverse effects. There was no change in oxygen saturations during care. Similarly, there was no difference in infant development (Bayley Scales of Infant and Toddler Development with the cognitive composition score; motor composition score; and the language composition score). Parents showed no difference in the incidence of anxiety or depression.

Evidence from Non-RCTs

Kumar et al.[41] examined the impact of music therapy for feto-maternal monitoring in nine pregnant women. They found that fetal HR increased from 146 to 169 beats per minute after exposure to music from headphones paced around the mother's abdomen. The increase in HR was consistent with a reactive NST. Music could serve as a means of communication with the fetus through sounds and voices. Caressing the fetus through the abdomen, producing soft and melodic sounds, using lights and vibrations were pleasant for the fetus.

In a prospective observational study in 100 uncomplicated pregnant women, between 32 and 41 weeks gestation, Erkun Dolker and Basar showed significant differences between the music-exposed and control groups in terms of mean numbers of acceleration, deceleration, and reactive NST results ($p = 0.001$).[192]

Gebuza et al.[193] showed a significant reduction in the number of uterine contractions, increased FHR accelerations and variability and increased fetal movements following exposure to classical music during NST in 48 pregnant women in their third trimester. Their recent study involving 30 (classical music during NST) and 60 (NST only) pregnant women between 27 and 41 weeks of gestation, confirmed their previous findings that classical music therapy could be a non-invasive, affordable method to improve fetal well-being.[193,194]

A Polish study showed a positive correlation between uterine contractility, fetal movements, and increasing music rhythm. There was no influence on fetal HR.[195]

Lee et al.[196] reported that musical intervention on fetal HR altered fetal Doppler between 30 and 38 weeks' gestation. Mean (±standard deviation) fetal movements during fixed singing activity (0.7 ± 0.79) were lower than during irregular singing (1.73 ± 1.37). This showed that fetal movement and HR could be stabilized by singing fixed songs to promote well-being and establish maternal–fetal bonding.

Future Directions

We still need more evidence to ascertain the precise role of timing of prenatal sound simulation. Methodologically strong RCTs involving this concept with rigorously designed interventions, consistent outcomes, and standardized reporting measures are needed. Future studies may include comparisons of different durations and types of musical (sound) intervention. Future studies can focus on specific effects of different types of music including instrumental music compared with vocals, classical vs metal and maternal vs other voices. It has been shown to promote maternal–fetal bonding.[49,79] Studies are needed for better understanding of the mechanisms by which music affects the growing brain, intrauterine neurosensory organization, and changes during development.[195] Changes to the physical environment, like darkening the room and decreasing visual and auditory stimuli in the infant's surroundings.

Music therapy may also be useful in specific maternal/fetal conditions such as drug dependance given the beneficial effects of music therapy in infants with neonatal abstinence syndrome postnatal conditions. Neonatal abstinence syndrome is potentially an important area for study; the numbers in the United States are staggering.[197,198] The incidence of NAS in the US started increasing in 2000 and nearly doubled between 2010 and 2017.[199,200] We need carefully conducted studies to determine the prenatal impact of maternal substance use on the fetus.[201]

Conclusion

Prenatal sound stimulation including music and mother's speech influences the development and function of the fetal/neonatal neurological system. Fetuses seem to respond to various forms of music that they have been exposed to. Further studies can be beneficial.

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REVIEW ARTICLE

Navigating Information Overload on Social Media: Opportunities and Misadventures for Clinicians and Professionals

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Abstract

In an age with numerous online and social media platforms where families, clinicians, and professionals rely more on the internet and social media for information finding, sharing, understanding, and analyzing medical literature is more complex than ever. Navigating social media has become increasingly difficult with misinformation and disinformation concerns and the growing number of dissemination methods. Social media plays an ever-increasingly significant role in facilitating or hindering the practice of evidence-based medicine. There are ever-growing challenges to practicing medicine in the age of social media.

This article describes the benefits and risks of using social media for health professionals to stay updated with medical literature and provides best-use practices for critical appraisal, specifically for adapting essential techniques of appraisal when encountering social media. This article also describes best-use practices for understanding and collaborating with colleagues and partnering with families in information sharing.

Keywords: Critical appraisal, Evidence-based medicine, Health and communication, Health and media, Social media, Misinformation.

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Introduction

Since the creation of the modern internet, social media has transformed human interactions. Billions of users all over the world utilize social media for connectivity, and its utilization has evolved over the last 25 years.[1,2] It started with local country-specific platforms that only allowed for profile creation and connectivity with known contacts; it has now evolved to creating personal and professional profiles with additional features like blogging, media sharing, instant messaging, and content creation on global platforms.[2] Businesses, corporations, and government entities have also leveraged international social media promotion, advertisement, and crowdsourcing.[2,3] Most individuals who utilize the internet use some form of social media, which has, in turn, transformed how patients obtain health information and communicate with their healthcare providers.[3–5]

Health information is disseminated in many ways, including traditional print articles, online resources, and social media platforms. Several social media platforms are now available that contain health-related content. Each portal differs depending on its structure and underlying algorithms. In addition, new ones are being formed frequently, and existing platforms are constantly evolving and being modified. Social media has increased social support, information sharing, and interconnectivity among individuals, families, and providers and provided an additional public health and health promotion platform.[5] The use of social media within medical education and clinical practice has also grown over the last few decades due to its availability, portability, ease of dissemination, interactivity, and ability to collaborate.[6] Many medical societies and academic journals have embraced social media by having professional accounts that distribute information, provide publication alerts, organizational promotion, and clinician and patient education.[7] Furthermore, clinicians and professionals frequently use social media as an adjunct for networking and professional education.[7] Previous surveys have described that 70–90% of physicians use social media for personal or professional networking and information aggregation.[8–10] Social media can play an important role in facilitating or hindering the practice of evidence-based medicine, particularly with clinicians becoming aware of the evidence. Evidence-based medicine frameworks include awareness, critical appraisal, assessment of trade-offs, application

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of the evidence, and evaluation of performance.[11] However, the increasing use of social media within medicine has resulted in the increasing dissemination of misinformation and equity issues of health information access for those without the internet.[5]

Given the rising use of social media within healthcare, this article describes how medical information is generated and disseminated across social media, how health professionals can evaluate the completeness and validity of evidence they encounter, and considerations for the best methods of social media use.

Content Generation and Consumption

Medical content on social media often comes from many sources. Primarily, traditional scientific content generally comes from journal articles or academic institutions. Secondarily, other sources could range from personal opinions, press releases, magazines, blogs, and health advocacy groups to government health policy statements and everything in between. Irrespective of the source, how this information is generated and uploaded on social media is very diverse. Content may be generated from a source journal, a server ("bot") that automatically publishes every new article in the field, or from individuals or groups that post their own or other people's work on various platforms. Generally, given character or page limits, information will likely be packaged in short "sound bites".

Often, there are heavy biases introduced because of how information is presented. For example, positive and sensational studies with a "clickbait" feel perform well and often receive greater dissemination through more likes, reposts, and shares. Once information is shared, several biases are introduced based on how users interact with the content. These include interaction bias, self-selection bias, and sampling bias. All of these compound into second-order biases, which feed into the social media algorithms.[12] Social media algorithms possibly produce the biggest bias by significantly influencing what users see and interact with on various platforms. Posts are elevated based on who posted it, their popularity (influencer, number of followers), the type of post (e.g., text, pictures, video, audio), and timeliness and timing of the post (recency).[13] The built-in algorithm is unique to each social media platform (and is constantly being revised) and determines how the posts are recirculated and appear on users' feeds. These include factors like how users interacted with posts (likes, reposts, shares, replies, comments, tagged, etc.). Platform developers update these algorithms regularly to ensure the currency and popularity of feeds. For example, what most celebrities or influencers post is likely to be read and consumed by the maximum users, and healthcare posts are not immune to this.[14] Given the biases and metrics involved, even if an individual does not follow an influencer in their field, because of the algorithm, their post will likely be circulated into the feeds of all users. In other words, social media posts may be missed based on how frequently they check social media and what the algorithm exposes them to.

With the increasing use of generative AI, including but not limited to large language models, the volume and quality of auto-generated and/or curated posts across platforms is also on the rise. The accuracy and authenticity of any post need to be carefully considered by the consumers.

Building a Health Professional Community or Network

Critical to the success of leveraging social media as a tool for translating evidence-based medicine into evidence-based practice is the use of networks and peers to build a community of practice based on integrity and trust. As previously detailed, much of the content generation for an individual consumer depends on the community they have built. Networks on social media can also serve as powerful tools for disseminating and critically evaluating evidence being presented.[15] Therefore, the social network one chooses to interact with directly significantly impacts the content being generated.

Communities of practice have been previously described as groups of people who share a common focus and improve their practice through interactions within the community.[16] The concept has been applied to social media networks to bridge the long gap between evidence-based medicine and clinical practice.[17] For example, the formation of a community of practice was described in the neonatal community on the social media platform Twitter (now "X"), formed around the hashtag #neoEBM and later with #neotwitter.[18,19] The pediatric intensive care unit (PICU) community also demonstrated how social media could be a powerful tool for dissemination during challenging times, such as the COVID-19 Pandemic.[20]

A community of practice connected through social media can offer several advantages for disseminating evidence-based medicine. The annual growth of journals and publications each year makes it difficult for a single person to keep track of clinical research, even if they stick to their specific area of expertise.[21] A community of practice can effectively "crowdsource" this information, taking a burdensome task for a single person and dividing it into the community. Free and immediate access to colleagues through an online community also allows for novel ways to communicate about emerging research. For publication authors, social media provides a unique tool to add context and clarity or educate peers in ways that do not fit publication requirements. Social media can replace or enhance the traditional methods of correspondence (e-mail, letters to the editor) with direct access to study authors for clarification or critical discussion. A large, widespread community of practice can also provide a quick means of assessing the generalizability of the study by leveraging the broad reach of the community to gain a brief glimpse into practice variation.

Along with these potential benefits come some concomitant challenges that require a thoughtful, measured approach to utilizing social media to complement evidence-based medicine. Research studies that are shared through social media can be of varying quality, dependent on the process of selection applied by the person sharing the content. As mentioned, automated bots search online databases for keywords and share content without critical appraisal. This differs from experts with a background in evidence-based medicine sharing content through social media after critically assessing an article. There is also a natural tendency towards self-promotion on social media, which can influence the dissemination of medical research. Spin in communicating results of mainly negative studies and information through publications and news has been prevalent.[22,23] While there are no published reports of the prevalence of spin through social media dissemination, it may be magnified without the filter of journal editors and peer reviewers. An evidence-based community utilizing social media should encourage thoughtful, balanced critical appraisal, allowing an open discussion of both the strengths and limitations of study findings. When building a social media network, it is important to be aware of these limitations, identify trusted sources of dissemination, and always conduct your own critical appraisal without taking study findings shared on social media at face value.

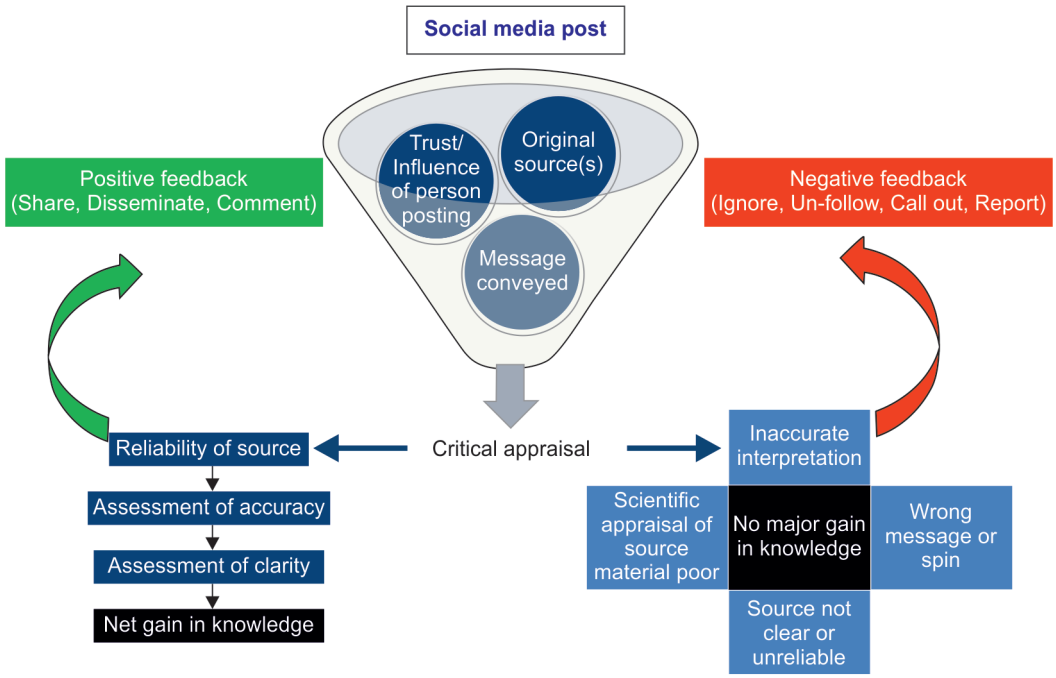

Fig. 1: Critical appraisal and dissemination of social media

Critical Appraisal

Critical appraisal is a systematic process of evaluating research for validity, importance, and applicability. Social media has no filters and triage mechanisms that medical journals utilize to cull flawed evidence, such as editorial rejection, peer review, revision, editorial oversight, retraction, expert commentaries, and post-publication peer review. Therefore, clinicians should develop a habit of critical appraisal when exposed to information on social media. Clinicians should consider obtaining and reviewing the complete article, placing it in the context of prior literature, and assessing the clinical expertise, all within the context of patient values and preferences. This will aid in deciding whether to incorporate the evidence into practice. Traditional approaches to critical appraisal include evaluating the research based on the presence of a clear research question, study design and implementation, design biases, confounders, and appropriate interpretation of results and conclusions.[24] Applying critical appraisal techniques when reviewing social media can help clinicians avoid incorporating inaccurate or incomplete information into practice.

Systemically appraising social media similarly involves evaluating the source, message, and interpretation, which can provide feedback on the accuracy or inaccuracy of the post. After critical appraisal, content can be shared with personal evaluation and opinions with other social media users or members of an online community of practice (Fig. 1). Admittedly, this ideal critical appraisal process requires time, access to the full text of articles, skills in study evaluation, and awareness of statistical methods and tests. Social media facilitates the grouping of users into 'echo chambers' or those with similar mindsets, and this can be an additional barrier to fair and transparent evaluation and dissemination of evidence.

Best Practices

With social media being all-pervasive in our daily lives, healthcare professionals often ask, "How can I apply social media to my practice?" Social media can be used in several ways by healthcare professionals, that include, but are not limited to, the following: (A) learning; (B) teaching health education to medical professionals, the public, as well as health advocacy; (C) professional networking; and (D) interacting with patients and families.[10] Depending on the degree of intended engagement, the time commitment for each domain could be substantial. Therefore, it is important to define one's goals for using social media before starting to use it.

Learning

This is perhaps the most common reason for trainees to engage in social media, as it transcends geographic and often financial boundaries and makes educational resources accessible. However, given the need for vetting of authenticity, there is always a risk of obtaining inaccurate information. Therefore, healthcare professionals should rely on social media channels from established organizations such as the New England Journal of Medicine, the American Academy of Pediatrics, the Cochrane Database of Systematic Reviews, etc., for their learning needs. Organizations and medical societies like these utilize their channels to dispel misinformation, discuss important topics and highlight landmark research.[25,26] One should trust individual social media accounts or those from freelance organizations only when trusted peers have thoroughly vetted them.

Clinicians often rely on their social media feed to keep themselves abreast with the latest research evidence. While social media is an excellent tool to keep oneself updated on new evidence, it is crucial to keep in mind that the bite-sized summary of research findings posted on social media, either by authors, peers, social media channels of journals, or academic organizations, may not always align with the magnitude or certainty of the corresponding evidence. To promote their research, researchers may deliberately or unknowingly introduce "spin bias" into their research communication, defined as "the intentional or unintentionally distorted interpretation of research results, unjustifiably suggesting favorable or unfavorable findings that can result in misleading

conclusions".[27,28] Therefore, clinicians should be aware of their own confirmation biases and consistently critically appraise the literature themselves or by expert peers before trusting and applying social media summaries to their practice. For up-to-date evidence, social media consumers should continually reevaluate their network and the sources they follow and adjust the sources they obtain information from to focus on high-quality, accurate information.

Teaching

Social media is an excellent tool for providing education that genuinely upholds the principles of equity, diversity, and inclusion, as geographic boundaries or unaffordable paywalls do not restrict this medium. However, ensuring the quality and consistency of the teaching content requires significant time commitment. Several online resources help guide clinicians in generating teaching content on social media. The following general principles may help to ensure effective teaching on social media: (A) maximize the use of visual aids such as infographics and short video clips; (B) engage the audience through questions and (C) online polls.

Professional Networking

Social media can be an excellent avenue to build professional networks, at any stage of one's career, especially in an age with increasing awareness about one's carbon footprint, thereby limiting in-person interactions. Professionals may use platforms like LinkedIn or Research Gate to share their credentials, areas of expertise, and professional accomplishments. In addition, joining healthcare-related online groups and attending virtual events may further help one connect with individuals with shared professional interests. However, one should keep their professional and personal social media accounts separate; the latter can be used to share personal stories and pictures with friends, family, and acquaintances.

Interaction with Patients and Families

Clinicians may use social media platforms to engage with patients and families in several ways, such as imparting health education, promoting healthy behaviors, and sharing other health-related information (e.g., access to specialized care).[29] However, clinicians must adhere to the following best practices while engaging with patients and families to ensure professionalism.[26,30,31]

- Maintaining patient privacy should be the top priority. Any identifying information, including pictures and videos, should not be shared without permission. Reviewing institutional social media guidelines before engaging in social media is advisable.
- Promote healthy behaviors in an evidence-based manner, i.e., aligning the language of recommendations with the level of evidence.
- Maintain transparency about potential conflicts of interest.
- Maintain professional boundaries (i.e., avoid engaging in personal or romantic relationships with patients).
- Show empathy and practice active listening.
- Maintain respectful and inclusive communication–avoid discriminatory or offensive language.
- Avoid specific medical advice on an open social media platform.

Conclusion

There are many opportunities for clinicians to use social media within medicine, including education, networking, and patient-family interactions. For optimal use, users should be aware of biases that impact content dissemination and understand how to leverage social media to build a community of practice.

Declaration

The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or US Government.

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CASE REPORT

Intrauterine Acquired Congenital Herpes Simplex Virus Infection in a Newborn

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Abstract

Aim: We present a fatal case of congenital herpes simplex virus (HSV) infection following exposure of a non-immune mother by her partner during critical fetal development.

Background: Globally, neonatal HSV infection affects 1 in 10,000 births. The most common mode of transmission is perinatally through passage through the vaginal canal (85%), followed by postnatal acquisition (10%). Rarely, intrauterine infection can occur (5%) resulting in congenital HSV, presenting with a classic triad of skin desquamation, chorioretinitis and brain malformations including hydrocephaly, anencephaly, and porencephaly.

Case description: A 30-week pregnant woman with a history of flu-like illness at 18 weeks presented with decreased fetal movement and vaginal bleeding. Initial evaluation showed echogenic bowel on ultrasound. At 30 weeks, polyhydramnios and fetal brain abnormalities were noted. A c-section was performed, and the infant required resuscitation. The infant's father reported a history of genital HSV outbreaks and HSV-2 was detected in the infant's blood. The infant had extensive skin desquamation, seizures, and succumbed to fatal brain malformations.

Conclusion: While maternal treatment with antiviral medication and cesarean section are effective in preventing perinatal HSV infection, congenital infection in the first or second trimester of pregnancy with devastating consequences for the fetus can occur in women without HSV immunity.

Clinical significance: Given the lack of available HSV immunization, protection of non-immune pregnant individuals from HSV exposure is currently the only preventive measure against congenital HSV disease.

Keywords: Brain malformation, Case report, Congenital infection, Herpes simplex virus, Newborn, Prevention.

Newborn (2024): 10.5005/jp-journals-11002-0109

Background

Herpes simplex virus (HSV) is a ubiquitous virus with an estimated global prevalence of 66% for the HSV-1 and 13% for the HSV-2 serotype.[1,2] Both serotypes can cause genital infection and have been implicated in neonatal HSV disease. Neonatal HSV can occur from either primary infection during pregnancy (25–60% risk of transmission), or rarely from an outbreak in a mother with a history of recurrent genital lesions (<2%).[3] Although both serotypes can cause neonatal HSV, serotype 2 has been linked to worse central nervous system (CNS) disease in infants.[4–6] Maternal diagnosis can be challenging since up to 80% of women are asymptomatic,[7] which delays treatment until the infant is recognized to be affected. Here we present a challenging case of an infant with rare intrauterine HSV-2 infection resulting in severe neonatal disease.

Case Description

A pregnant woman presented at 30 weeks gestation with decreased fetal movement, vaginal bleeding, and contractions. Pregnancy was notable for a flu-like illness at 18 weeks gestation with associated dysuria and incontinence. During that time, she passed a thick clump of mucousy blood-tinged discharge. A cervical exam was significant for a friable cervix with yellow frothy discharge. Urine culture was negative. Wet prep contained polymorphonucleated cells but otherwise unrevealing. Ten days following evaluation for the illness at 18 weeks, fetal ultrasound was notable for echogenic bowel with no other noted anomalies (Fig. 1). A standard HSV IgG laboratory panel revealed the presence of HSV-2 IgG [9.49 gm/L (normal 0.00–0.90)] but no HSV-1 IgG [<0.91 gm/L (normal 0.00–0.90)]. No testing for HSV IgM was performed. Given isolated echogenic bowel findings, she was recommended to have a routine follow-up with her obstetrician.

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Patient consent statement: The author(s) have obtained written informed consent from the patient for publication of the case report details and related images.

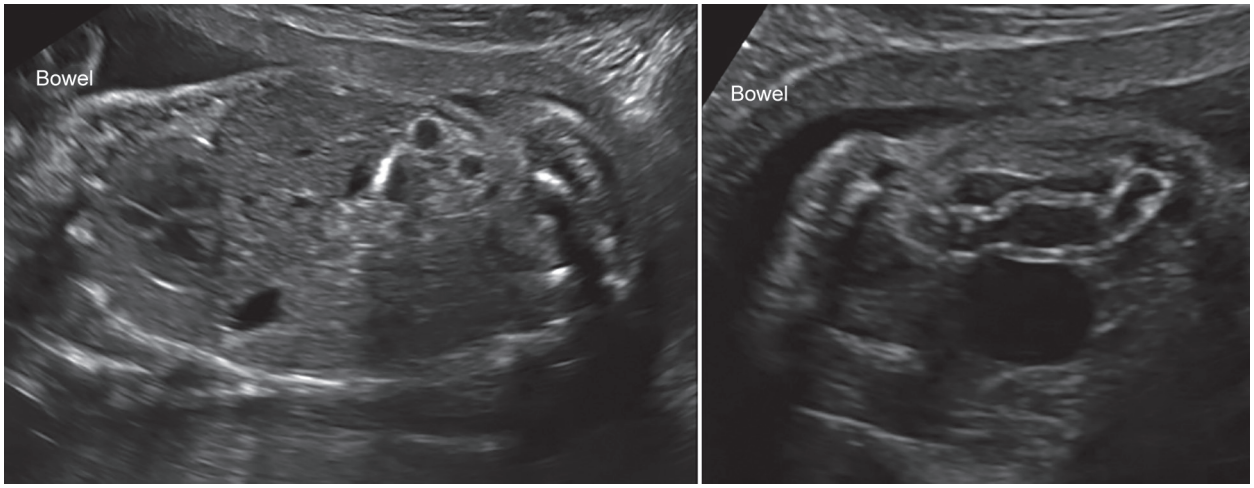

Fig. 1: Prenatal ultrasound showing portions of the fetal bowel to appear echogenic and fluid-filled. There are no signs of overt dilation, with the widest diameter measuring 6 mm (normal <7 mm)

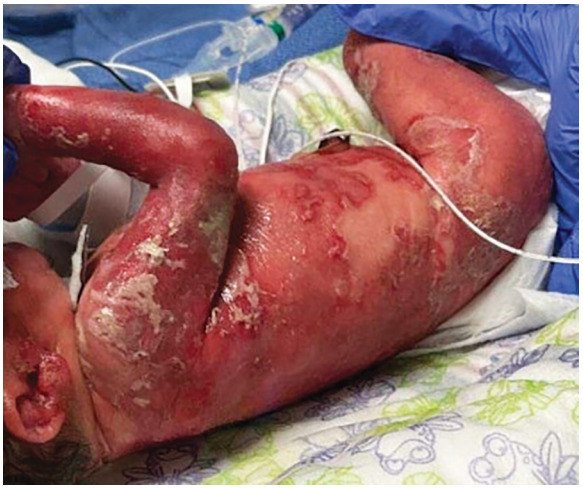

Fig. 2: Extensive desquamation and excoriation of right upper extremity and torso

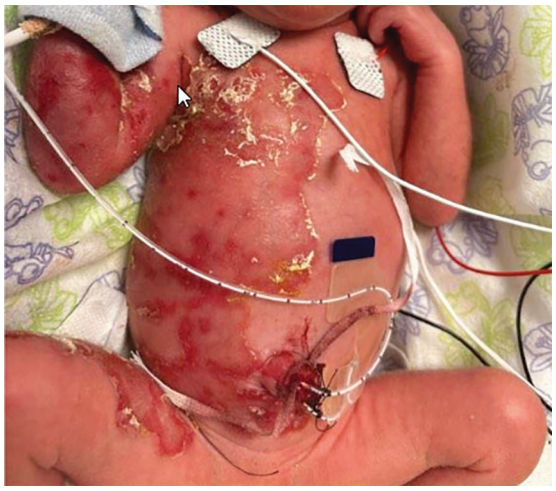

Fig. 3: Erythematous erosions and scarred thin plaques with some overlying crust in a dermatomal distribution along unilateral chest and trunk

On the day of the presentation, fetal ultrasound showed new findings of severely dilated 3rd and 4th ventricles, polyhydramnios, and a category II fetal heart rate tracing. Given these findings and a breech presentation, c-section was performed. At birth, the infant had poor tone with no respiratory effort. She was resuscitated following Neonatal Resuscitation Program (NRP) guidelines and intubated for apnea. Physical exam was significant for extensive skin desquamation involving the right arm, face, and torso (Figs 2 and 3). Her birth weight was 1,415 gm (60.9%, $Z = 0.28$), her head circumference 27.2 cm (50.1%, $Z = 0.08$), and her length 39.5 cm (62.8%, $Z = 0.33$).

Shortly after admission to the Neonatal Intensive Care Unit (NICU), the father of the infant provided a history of recurrent genital HSV outbreaks, with an episode shortly before the maternal febrile illness at 18 weeks of pregnancy. Polymerase chain reaction (PCR) testing of the infant detected HSV-2 from surface swabs of a skin lesion, the eyes, and blood. Toxoplasmosis IgG and IgM antibodies were negative, as was blood PCR for cytomegalovirus (CMV). The bacterial and fungal blood cultures showed no growth. Treponemal IgG was negative. Complete blood count was significant for thrombocytopenia to a nadir of 65×10^3 mc/L and C-reactive protein was 2.6 mg/L (0.3–6.1 mg/L).

Given the desquamating appearance of the rash, the differential diagnosis included epidermolysis bullosa or bullous pemphigoid, although this was moved lower on the differential once it was appreciated that the lesions did not extend from skin trauma. An infectious etiology such as disseminated candidiasis was considered especially with white demarcation of plaques. Infections, such as CMV, toxoplasmosis, rubella, syphilis, parvovirus, and HSV were considered in the setting of prenatal brain malformation.

Prompt treatment with ampicillin, gentamycin, acyclovir, and fluconazole was started. Subspecialty involvement of

Dermatology recommended sterile water baths and generous use of emollients. Ophthalmologic evaluation was significant for bilateral acute retinal necrosis without retinal detachment, as well as corneal dendrites on the right (Fig. 4) and corneal abrasion on the left. The patient was started on artificial tears, ganciclovir, and moxifloxacin eye drops. Shortly after admission to the NICU, the patient developed right arm rhythmic jerking concerning seizures. She was placed on continuous electroencephalogram (EEG) which confirmed seizure activity and an abnormal background consistent with lack of cortical electrical activity except for ictal events. A head ultrasound revealed poor differentiation between gray and white matter, ventriculomegaly of the lateral and third ventricles with effacement of the fourth ventricle, an absent corpus callosum, punctate foci of echogenicity near the left vertex concerning ischemia vs hemorrhage, a cystic structure in the posterior fossa with mass effect on an atrophic appearing cerebellum, and diffuse periventricular echogenicity (Fig. 5). Given all these findings and severe neurologic prognosis, her parents made the loving choice of redirecting her care and allowed natural death.

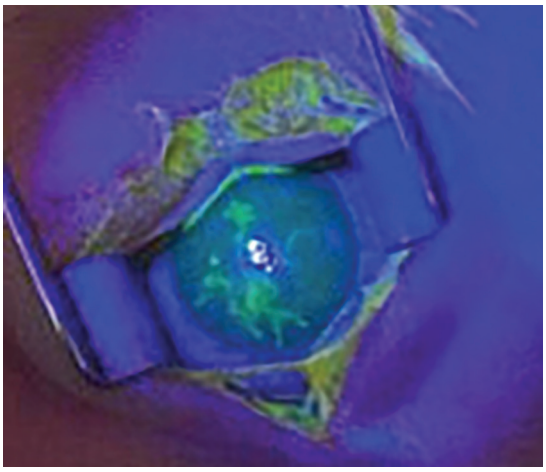

Fig. 4: Fluorescein staining of right eye showing large dendritic lesion

Discussion

Although rare, in utero HSV transmission can present with severe devastating consequences for the fetus as demonstrated here. Thirty percent of in utero infections occur in the first trimester, 30% in the second, and 40% in the third.[8] In a literature review by Fa et al. of 36 congenital HSV cases, the most common postnatally observed manifestations are skin desquamation, chorioretinitis, and hydrocephaly, which were all features of this case.[9] Neonatal skin findings are heterogenous and include erosions, plaques, erythematous patches, or ulcerations. Atrophic scars and aplasia of the skin have been described and despite the neurotrophic characteristics of the virus, dermatomal distribution of the skin lesions is uncommon.[10]

Prenatal recognition of HSV infections can be challenging. Maternal primary genital HSV-2 infection can present with a febrile illness and flu-like symptoms along with vaginal or cervical lesions,[9] but over 80% of women are asymptomatic.[8] Thus, most cases of neonatal herpes occur in mother–infant dyads in which the mother is undiagnosed.[8,11,12] Prenatal ultrasound findings of congenital herpes infection involve primarily the CNS and are non-specific, creating a broad differential diagnosis. The CNS abnormalities associated with congenital HSV include ventriculomegaly, hydrocephaly, agenesis of the corpus callosum, porencephaly, intracranial calcifications, cerebral hemorrhage, microcephaly, and microphthalmia.[9] Herpes simplex virus-2 has been observed to present with more severe CNS findings compared with HSV-1.[3] The risk of transmission to the fetus or newborn is much greater with a primary genital infection during pregnancy compared with reactivation of infection (25–50 vs 1%),[11] although congenital HSV-1 has been reported in mothers with primary gingivostomatitis febrile illness.[3,13] Up to 50% of cases of neonatal HSV are due to the HSV-1 serotype, with 75% of those associated with recently acquired genital HSV-1.

Fetal HSV transmission resulting in congenital HSV infection is thought to occur from maternal viremia and passage through the placenta. Placental pathology in this case showed negative staining for the herpes virus. This is likely due to the temporary shedding of the virus during the acute illness, which again highlights the high index of suspicion needed for the correct diagnosis and treatment of HSV infections. Placental pathology was significant

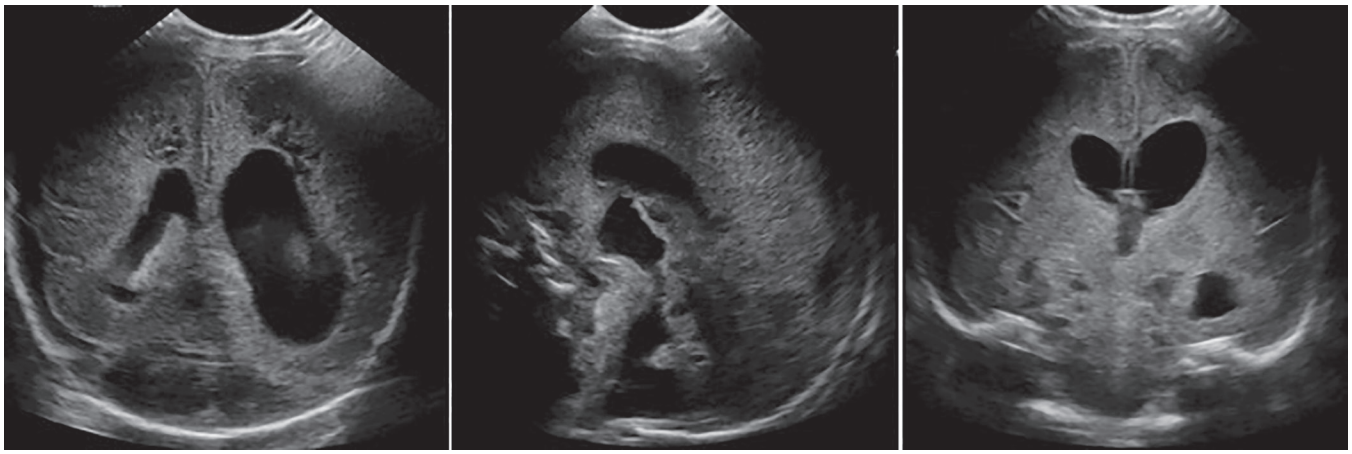

Fig. 5: Head ultrasound images showing poor differentiation between gray and white matter, ventriculomegaly of the lateral and third ventricles, an absent corpus callosum, punctate foci of echogenicity near the left vertex concerning for ischemia versus hemorrhage, and a malformed cerebellum.

for accelerated villous maturation, a three-vessel cord with acute vasculitis, organizing intervillous hematoma, and multifocal villous edema, which have all been described in the literature.[14] Infant postmortem autopsy was not performed per parental request.

Pregnancy may increase susceptibility to new infection. Prevention of primary infection during pregnancy currently includes barrier protection when sexual partners are known to be seropositive for HSV and treatment of the infected partner with daily valacyclovir. Condoms and valacyclovir each confer around a 50% rate of efficacy in the reduction of transmission of genital HSV. Measures taken to reduce congenital herpes include the administration of antivirals for women presenting with a primary HSV infection during pregnancy. Treatment at the time of diagnosis decreases viremia and transmission.[15] Antiviral therapy is also recommended for women at or beyond 36 weeks of gestation who are at risk for recurrent HSV infection. Cesarean delivery is recommended for women with active primary or secondary HSV lesions at the time of delivery.[16]

Universal screening with serologic testing in pregnancy has been proposed to identify the women at the highest risk for primary infection during pregnancy. Not only would this approach detect women at risk for infection, but it would identify women with existing asymptomatic infection at risk for viral shedding at delivery.

Conclusion

Although rare, in utero HSV transmission can have devastating consequences for the fetus. While maternal treatment with antiviral medication and C-sections are effective in preventing perinatal HSV infection in the newborn, limited options currently exist to prevent congenital infection in the first or second trimester of pregnancy.

Clinical Significance

While current research is underway to develop monoclonal antibody treatments to reduce recurrent infection and perinatal transmission,[17] vaccine development offers the most effective strategy to prevent or reduce herpes infections.[18] While prophylactic vaccines would need to be administered in early childhood, therapeutic vaccines could decrease shedding and reactivation of HSV in symptomatic and asymptomatic adults and therefore lower the infection risk for susceptible pregnant women.[2]

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CASE REPORT

Neonate with Bilateral Vocal Cord Palsy Presenting with Respiratory Distress and Congenital Stridor: A Diagnostic and Therapeutic Challenge

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Abstract

Objective: We recently treated a neonate with biphasic stridor secondary to bilateral vocal cord palsy (BVCP). This experience evoked considerable discussion in our unit; hence, we have outlined our approach to neonatal stridor, the importance of direct visualization using bronchoscopy, and management options in this condition.

Case presentation: A full-term male infant presented with biphasic stridor two days after birth. The pre- and peri-natal course was uneventful, but he developed respiratory distress immediately after birth and needed assistive ventilation. There was no remarkable lung disease; the radiographs were reported as normal. We were able to wean him to non-invasive respiratory support within 48 hours, but there was persistent biphasic stridor with increased work of breathing. Extensive evaluation of the airways using flexible and rigid bronchoscopy showed BVCP. There was no change in the vocal cord movement over time, and eventually, on day 38 after birth, we had to perform a tracheostomy. He was successfully discharged home after a few days. So far, after a few months, he continues to tolerate feedings and has shown good growth, but there has been no change in BVCP.

Conclusion: Vocal cord palsy should be considered as a possibility in infants who present with stridor and respiratory distress but have a noticeable cry. Transnasal fiberoptic flexible laryngoscopy is an important tool in assessing and monitoring these infants. A comprehensive evaluation should ascertain whether the laryngeal dysfunction is an isolated, primary clinical problem or part of a secondary systemic infectious/syndromic illness. The prognosis will depend on the etiology; isolated vocal cord palsy usually takes months to years to show improvement, so surgical treatment options may have to be explored. In contrast, secondary laryngeal paralysis will need more extensive systemic assessment, monitoring, and prognostication; treatment focused on cure, remission, or rehabilitation might be possible in some infants based on the specific diagnosis.

Keywords: Bronchoscopy, Case report, Newborn, Respiratory distress, Stridor, Tracheostomy.

Newborn (2024): 10.5005/jp-journals-11002-0110

Key Points

- We recently treated a neonate with biphasic stridor secondary to bilateral vocal cord palsy (BVCP). The etiology could not be determined even after extensive evaluation.
- The infant was admitted to our neonatal intensive care unit two days after birth with increasing stridor and respiratory distress. The evaluation for infections and lung diseases was unremarkable.
- Evaluation using flexible and rigid bronchoscopy showed BVCP. There was no evidence of infections or syndromic disorders.
- There was no change in the clinical condition over time. On postnatal day 38, a tracheostomy was performed to enable a safe discharge from the hospital. A follow-up program was designed with home pulse oximetry, feeding monitoring, and frequent growth and developmental assessments.

Background

In this report, we have summarized our recent experience with a full-term infant who was admitted with respiratory distress but all the other systems showed normal function. Once intubated, the ventilatory needs were minimal. There were no dysmorphic features or other systemic abnormalities. When extubated after 48 hours of a stable clinical course, he developed stridor and respiratory distress. The evaluation showed bilateral vocal cord paralysis (BVCP).[1]

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Vocal cord paralysis with laryngomalacia is a rare disease with an incidence of 0.75–2 cases per million births per year.[2,3]

In terms of etiology, the condition can be neurogenic, part of a systemic infectious or syndromic diagnosis, or be idiopathic.[4] The management depends on the etiology and severity; the options include a careful conservative follow-up, a tracheostomy, an endoscopic posterior cricoid split with cartilage grafting, or an endoscopic anterior and posterior cricoid split with dilation and stenting.[5–7] The conservative follow-up appears safe but many of these patients show suboptimal growth and development, possibly due to the persistently high work-of-breathing.[8] In idiopathic BVCP, similar to most rare disorders, the choice of the treatment option(s) is usually influenced by the care provider (s) intuition, past clinical experience (if any), and shared clinical decision-making process following a group discussion.[9] Not surprisingly, the least invasive treatment options are chosen most frequently. There is a commitment to frequent follow-up, which happens in many, but not all, patients.[10]

Case Presentation

A 28-year-old G_2P_1 mother with a planned spontaneous pregnancy gave birth to a 38 weeks' gestation/2,800 gm birth weight male infant by a spontaneous vaginal delivery. Other anthropometric measurements were within normal limits: the length was 51 cm, and the head circumference was 34 cm (all parameters were appropriate for gestational age based on Modified Fenton's charts).[11] This was a non-consanguineous pregnancy with no history of any medical or obstetrical complications; prenatal sonograms were unremarkable. Apgar scores were 9 and 9 at 1 and 5 minutes.

The infant developed respiratory distress soon after birth with marked tachypnea, chest retractions, and cyanosis. Given these findings, he was intubated and admitted to the neonatal intensive care unit (NICU). Other than the respiratory parameters, other vital parameters and activity were normal and remained stable. The rest of the physical examination was normal with no dysmorphic features.[12] He was intubated and was easily managed with low ventilatory settings. Venous blood gas, chest X-rays (CXRs), and 2D echocardiography were normal.[13–16] Blood counts were within normal limits.[8] After a few hours of confirmed hemodynamic stability he was started on milk feeds via a nasogastric tube.[13,17] The respiratory and circulatory parameters were monitored and remained normal. Blood cultures remained negative.

Two days after birth, he was extubated to respiratory support using a high-flow nasal cannula (HFNC) with heated, humidified air.[18] However, he developed severe biphasic stridor within a few hours and had to be re-intubated.[19] Once the respiratory status was stabilized, the CXRs again looked unremarkable. Given the stridor, we evaluated the upper airway during intubation. There was no relief with a change in positions, no pooling of secretions, or regurgitation of feeds.[20] The records were reviewed; there were no observations of any stridor or difficulties during intubation. A detailed examination by a clinical geneticist ruled out any other syndromic associations.[21–26] A cranial ultrasound and serum levels of calcium and magnesium were evaluated and found normal. There was no evidence of any congenital/perinatal bacterial or other infections (Toxoplasma, Rubella, Cytomegalovirus, Syphilis).[19] Parents tested negative for Coronavirus 19.[27] Subclinical Epstein–Barr virus (EBV) infection was considered but considering the low frequency of these infections in neonates and because appropriate tests were not available at our hospital, these were deferred to be performed if the infant were to show any signs of inflammatory disease.[28]

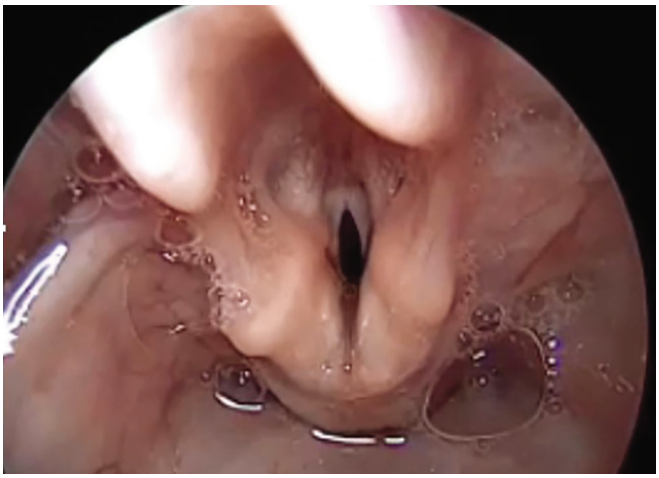

Fig. 1: Transnasal flexible bronchoscopy finding of bilateral non-mobile vocal cords confirming the diagnosis of bilateral abductor palsy

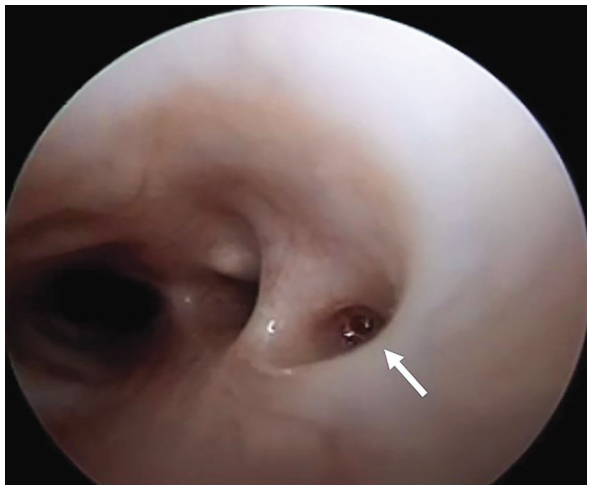

Fig. 2: Incidental finding of the additional bronchus (porcine bronchus) on rigid bronchoscopy

Considering the possibility of a congenital airway abnormality causing stridor, the infant was evaluated by the pediatric otorhinolaryngologists; a transnasal flexible fibreoptic laryngoscopy[29] was performed first, followed by a rigid bronchoscopy under anesthesia.[30] They noted bilateral non-mobile vocal cords confirming the diagnosis of bilateral abductor palsy[31] (Fig. 1 and Supplement 1). An incidental finding of a porcine bronchus (Fig. 2) was noted.[32]

The infant tolerated orogastric tube feedings well during the period while he was on respiratory support. We held periodic multidisciplinary meetings including the neonatologists, our pediatric otorhinolaryngologist, clinical psychologist, social workers, and parents to update all team members.[33] At the end of the first month, all growth parameters showed good incremental gains.[34] However, there was no improvement in the respiratory condition; the biphasic stridor was persistent but manageable using an HFNC with no/minimal oxygen. Given the increasing duration of the NICU stay, we evaluated him for a tracheostomy to facilitate discharge from the hospital. The team was concerned about the neurodevelopmental consequences of prolonged separation from

the family and the risk of nosocomial infections due to prolonged hospital stay.[35,36]

The family was not particularly keen on subjecting the infant to a simultaneous tracheostomy and gastrointestinal tube placement; we respected their decision as the outcomes of simultaneous or sequential procedures are not very different.[37] Many infants with BVCP can have esophageal dysmotility/swallowing dysfunction, which can also increase the risk of aspiration with age-related needs for larger feed volumes.[38,39] We did not have the option of esophageal manometry studies for neonates at our center.[40] There was a discussion about a radio-opaque dye-enhanced swallowing study but our team and the family were not particularly keen on performing these studies because they felt that it would not alter our decision-making; the infant anyways did not have clinically evident tracheoesophageal dyscoordination, major pulmonary aspirations, or swallowing dysfunction that would have been seen as gagging/vomiting, need for supplemental oxygen, or radiological changes suggestive of micro-aspirations.[39,41] The infant had a reasonable volume of sound in his cries, which was also encouraging. After considering all the pros and cons, a decision was made to place only a tracheostomy.[42] An incremental plan was chosen for surgical interventions, where a gastrointestinal tube placement/Nissen fundoplication would be considered later as a second procedure only if required.

A tracheostomy was performed on postnatal day 38 and an uncuffed tracheostomy tube size 3.0 was placed at tracheal cartilage level 2–3.[7] The procedure was uneventful and the infant was subsequently continued on HFNC support and tube feeds. Both parents remained actively involved in his care with help from assigned nursing staff. A very conservative feeding plan was made. On postnatal day 45, the HFNC was discontinued and he was transitioned first to cup and spoon feeds and then to breastfeeds. Once the team and the family were comfortable, he was discharged home on postnatal day 60 with a tracheostomy tube *in situ*.[43]

The infant is currently three months old. Parents have shown confidence in tracheostomy care at home. He has been accepting oral milk feeds without any obvious episodes of feed aspiration. Growth and neurodevelopment are within normal ranges. The surgical team has decided to reassess the possibility of decannulation at one year of age.

Discussion

Congenital laryngeal abnormalities are a major cause of respiratory distress including stridor in a neonate.[44] Vocal cord palsy (VCP) due to altered motor nerve supply of the larynx is the cause of stridor in 10–15% of these cases. The recurrent laryngeal nerve branch of the vagus, which regulates both the abduction and adduction of the vocal folds, is malfunctioning in about 20% of cases.[38] Most cases of unilateral VCP appear to be iatrogenic such as following cardiac surgery, but BVCP can be due to neurogenic causes such as perinatal asphyxia, hypoxic-ischemic encephalopathy, intraventricular hemorrhage, and hydrocephalus or syndromes such as Arnold Chiari malformation type I.[45–47] About a fourth of all cases, as in our index case, can be idiopathic.[38] Unilateral and bilateral VCP can differ in the clinical profile including the age of presentation, symptoms and signs, management modalities, and recovery.[38]

In the existing literature, the median age of presentation of VCP is around 81 days. Stridor is the most frequent presentation, seen in 96% of cases with BVCP and 77% of unilateral VCP.[48,49] More severe forms of VCP have also been noted; Bilateral vocal cord palsy can present with respiratory difficulties and apnea. Nearly 60% of all patients with congenital BVCP show spontaneous recovery.[48] In this context, our infant had an unusually early presentation after birth. In infants with stridor, timely consultation with pediatric otorhinolaryngologists is important. Flexible bronchoscopy can help evaluate vocal cord mobility in an awake neonate. If needed, a rigid bronchoscopy can be performed under anesthesia to rule out other associated congenital airway anomalies. In a unit audit of pediatric bronchoscopy (unpublished data) of 140 children less than two years with stridor done by one of the co-authors, there were four children with BVCP, four with unilateral VCP, and one with posterior glottis stenosis.

The goal of management in VCP is to maintain patency of the airways. Several therapeutic options are available based on the etiology and severity of the condition.[50] Spontaneous recovery is seen in 50–65% of patients at five months to three years of age. However, a surgical procedure might be needed more frequently in patients with BVCP, in up to 74% of patients.[49] Tracheostomy can be performed to bypass the restricted glottic airway in these patients; the timing may have to be decided on a case-by-case basis, but the procedure might be needed more frequently in infants with comorbidities such as Arnold-Chiari malformation, other neurologic conditions or concomitant airway disease. Decannulation is usually delayed until after infancy.[51] Bronchoscopy-guided evaluation of BVCP with multidisciplinary management and post-tracheostomy home care can optimize the outcomes in these patients.[38]

In our index case, we performed a tracheostomy on postnatal day 38. Considering that the etiology was uncertain and the presentation was in early infancy, an assessment of gastrointestinal motility could have been helpful. Esophageal dysmotility can add to the risk of aspiration of feedings before and following tracheostomy.[52] In infants with VCP, as mentioned above, additional evaluation for subclinical viral infections such as herpes simplex, EBV, and COVID-19 needs additional study. Inherited (autosomal dominant) and de novo mutations have also been implicated in VCP seen in older patients.[23]

Learning Points

- Vocal cord palsy should be considered as a possibility in infants presenting with stridor and respiratory distress.
- Transnasal fiberoptic flexible laryngoscopy remains the gold standard in diagnosing VCP.
- Vocal cord palsy usually improves spontaneously but may take years to resolve and when indicated, surgical treatment options should be explained.
- Timely intervention and consistent follow-up are essential in patients who have VCP to document airway stability and recovery of vocal cord function.
- In neonates with idiopathic VCP, further evaluation for unusual viral infections and genetic mutations should be considered.

Ethical Approval

Parents are well-educated and have performed extensive internet mining for information. They have been closely involved in monitoring, tracheostomy, and follow-up. They have clearly stated that no further ethical discussions are needed and welcome any feedback from clinicians/centers worldwide.

Supplementary Materials

All the supplementary materials are available on the website www.newbornjournal.org.

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