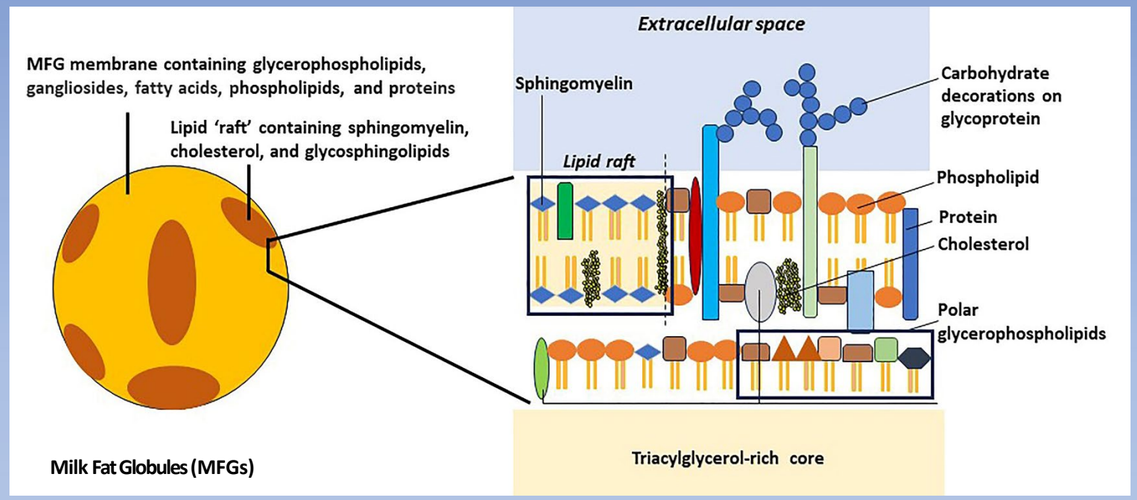
January–March 2024 Volume 3 Issue 1 newborn Official Journal of the Global Newborn Society eISSN: 2769–514X

newborn

January–March 2024
Volume 3
Issue 1
eISSN 2769-514X

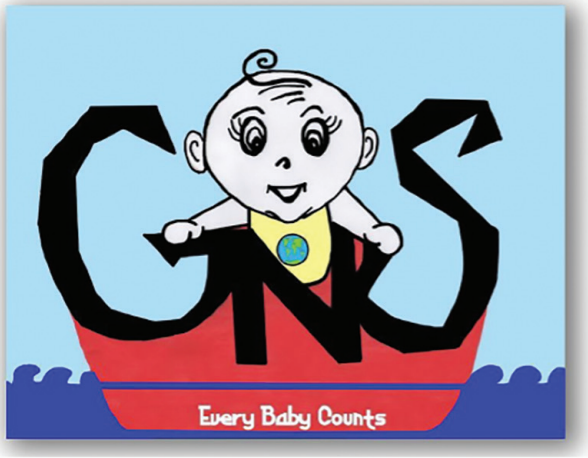
Official Journal of the Global Newborn Society
Official Journal of the GNS Down Syndrome Foundation
Official Journal of the Autism Care Network Foundation
Official Journal of the Newborn Foundation of Azerbaijan
Official Journal of the GNS Bangladesh Newborn Foundation
Official Journal of the GNS Foundation of Germany
Official Journal of the Global Newborn Society Foundation of Italy
Official Journal of the Mongolian Association of Obstetrics Gynecology and Neonatology
Official Journal of the Foundation for Human Milk Feeding in the Islamic World
Official Journal of the Sociedade Brasileira de Atenção ao Recém-Nascido
Official Journal of the organization, Protecting Brains and Protecting Future, Brasil
Official Journal of the Association of Neonatologists in the United Kingdom
Official Journal of the Polish Nursing Association - Płock, Poland
Official Journal of the Panlibyan Neonatal Association
Official Journal of the Association for Indigenous Peoples in India
Official Journal of the Association for Newborn Care in Pakistan
Official Journal of the GNS Association for Perinatal Care
Official Journal of the Association for Infant Nutrition in the Middle East

***Highlighted articles*:**



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. 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 graphically summarizes our thought-process. There is a lovable little young infant exuding innocent, genuine happiness. The curly hair, shape of the eyes, long eye-lashes, 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, world-wide 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 all hope of peace and tranquility is not completely lost yet. The acronym letters, GNS, on the starboard are our three pillars of strength. 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, review articles, consensus statements, guidelines, trials methodology, and outcomes relevant to fetuses//neonates/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. Manuscripts can be submitted via the online manuscript submission system.

Issue Information

Volume 3, Issue 1; January-March 2024
ISSN: 2769-514X

Published: GNS, LLC; 6114 Lily Garden, Clarksville, MD, USA; Ph +1 708 910 8729
Printed: Jaypee Brothers Medical Publishers
4838/24, Ansari Road, Daryaganj, New Delhi 110 002, India
Phone: +91 11 4357 4357, Fax: +91 11 4357 4314

Contents

10.5005/newborn-3-1-iv

To Save Babies, We Need a Team – Let's Build One!

All over the world, infancy is a period marked by a high risk of morbidity and mortality.[1-3] And a death so early in life has implications – each time we lose an infant, we lose an entire life and all its potential.[4] Advances in neonatal care have stimulated the development of multidisciplinary rehabilitation teams to provide supportive services for these patients.[5] All of us understand the goals of improving outcomes of infants and families, but the aims and objectives may need to be tailored for individual centers, communities, and even countries.[6–9] The panoramic truth is that to save babies, we need teamwork.[10–12]

In both high-acuity and/or limited-resource settings, we often need a wide array of medical providers, support services, and family members to interact dynamically and interdependently to care for newborn infants.[13,14] Higher the acuity of illness, greater might be the need for "fluidity" with adaptive team membership.[15] Based on philosophy, resources, and other considerations, the models of service delivery may have to be tailored for hospitals, communities, societies, and countries. We need to develop professional competence, collaboration, continuing education, effective communication, accountability, legislative support, and mutual respect that need to be focused on individual patients, families, and care units.[16–18]

Our journal, the *newborn* aims to cover fetal/neonatal problems that begin during pregnancy, start at the time of birth, or occur during the first 1000 days after birth. As in our previous issues, we present 8 important articles (**Figure 1**). In this 1st issue of the 3rd volume, we report three developments. Each one has the same message – to save babies, we need a team. *Let's build one!*

(a) Seventeen associations of care providers from all over the world are now collaborating with the Global Newborn Society (GNS) and have adopted the *newborn* as their official journal. In addition to the GNS, we are now the official mouthpiece for associations focused on Down syndrome, autism care, infant nutrition, and neonatal brain injury in infants from many countries, including (*East to West*) Bangladesh, India, Pakistan, Mongolia, Iran, Azerbaijan, Poland, Libya, Italy, Germany, the United Kingdom, Brazil, and the United States of America. One of the new participating associations spans the whole Islamic world. Could/should we say that what was once a small step for one baby is now evolving into a movement, possibly a longer leap for humankind?

(b) We bring to you the statement from the Joint European Neonatal Societies' (jENS) Congress that was held in Rome in September 2023.[19] Each year, we lose nearly 1.9 million fetuses in stillbirths and about 2.3 million newborns.[1,20] The vast majority of these deaths are recorded in the relatively disadvantaged peri-equatorial and tropical countries, and most are preventable. Many necessary, proven, and highly effective interventions are available but we will need to work together to improve usage/access to these save more babies and reach the 2030 Sustainable Development Goals (SDGs).[21] To reach the SDG target of <12 neonatal deaths per 1000 live births,[22] we need to invest smartly, implement sustainable programs, integrate our efforts, and innovate to improve the efficiency of these efforts;

(c) March 21st is the World Down Syndrome Day – a global awareness day that has been officially observed by the United Nations since 2012.[23] We definitely need lots of socks[24] to express our solidarity, but we also need more original thinking to help these babies. In this issue, we present a report that may have important implications for clinical care. The authors provided care to a term[25] male infant with persistent patency of the *ductus arteriosus*. His fetal tests had shown some ambiguity for trisomy 21 but there were no phenotypic features typical of trisomy 21[26] *in utero*[27] or after birth[28] and the postnatal karyotype was reported as normal.[29] A repeat test that got requested after birth by chance showed mosaicism[30] for this aneuploidy.[31,32] The mechanisms underlying the origin of trisomy 21 mosaicism are still unclear, and this report is a reminder that incidence of this condition in the community might be higher than our current state of knowledge. There is a need for further studies.

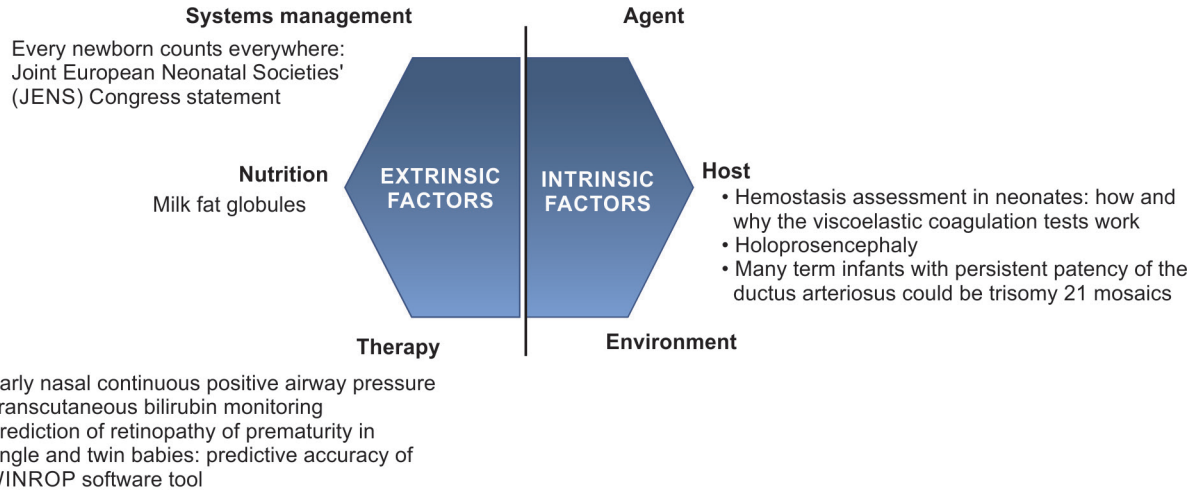


Fig. 1: **Areas of focus in the *newborn*, Volume 3, Issue 1.** We have expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three additional foci represent extrinsic factors that can affect health—those originating in therapy, nutrition, and systems management. This issue covers 4 of these foci, namely host factors, treatment/monitoring systems, nutrition, and systems management.

There are 3 important original studies in this issue. Mohan *et al*.[33] investigated the effectiveness of a software tool to screen retinopathy of prematurity (ROP), the WINROP (*Weight, Insulin-like growth factor I, Neonatal ROP)* in a retrospective single-center study. They studied a cohort of 63 patients born at a median of 30 weeks' gestation with a birth weight of 1250 g. Twenty-two infants developed type 1 and 39 developed type 2 ROP. WINROP alarm was triggered in 33 (52.38%) infants. Pregnancy-induced hypertension (PIH), malnutrition, respiratory distress syndrome, blood transfusion, and anemia of prematurity were associated with the detection of ROP. The sensitivity, specificity, positive predictive value, and negative predictive value of WINROP to predict type 1 ROP were 63.6%, 53.6%, 42.4% and 73.3%, respectively. In its current stage of development, the tool has modest accuracy, but it might be useful alongside clinical screening for ROP in infants. These findings justify continued efforts to develop newer software-based predictive tools.

In another study, Sahota and coworkers[34] compared transcutaneous bilirubin levels on a covered, defined spot of skin with serum bilirubin levels prior to starting, during, and 2 days after discontinuing phototherapy in preterm and term newborns. They studied 272 babies with neonatal jaundice. Transcutaneous and total serum bilirubin levels showed statistically significant positive correlation before, during, and after discontinuing phototherapy in both preterm and term newborns. These findings are important as transcutaneous bilirubin may be a good noninvasive tool for monitoring infants with hyperbilirubinemia.

Hameed *et al*.[35] investigated whether timely institution of respiratory support with nasal continuous positive airway pressure (nCPAP)[36] could improve clinical outcomes in a limited-resource region of Iraq affected by chronic conflicts. They followed 123 preterm infants born at 26–32 weeks' gestation in a prospective cross-sectional study over a period of 6 months. These infants were treated with nCPAP soon after delivery or at admission in the neonatal intensive care unit. Early CPAP was successful in infants who were born at ≥28 weeks' gestation with a birth weight ≥1500 g, had received antenatal steroids, and did not have a history of premature/prolonged rupture of membranes. These infants had mild-looking respiratory disease on radiographs. Some had evidence of sepsis. Treated infants required surfactant and mechanical ventilation less frequently. Fewer infants had pulmonary hemorrhage. There was a significant reduction in mortality in these infants.

We bring to you 3 reviews. One of these, which is highlighted on the cover of this issue, is focused on milk fat globules (MFGs).[37] Human milk (HM) contains 3–5% fat, 0.8–0.9% protein, 6.9–7.2% lactose, and 0.2% mineral constituents.[38,39] These nutrients are largely carried in MFGs composed of a triacylglycerol core enclosed in a triple membrane structure.[40] The membrane contains polar lipids, specialized proteins, glycoproteins, and cholesterol. MFGs release energy in the upper gastrointestinal tract and then persist for some time in the gut lumen so that the protective bioactive molecules are conveyed to the colon.[41] These properties may shape the microbial colonization and innate immune properties of the developing intestine. There might also be possible roles in enhancing neurodevelopment, insulin sensitivity; and suppressing chronic inflammation.[42] This review aims to update the readers about the composition, structure, and biological activities of MFGs.

In recent years, new approaches to neonatal hemostasis have been explored.[43] Most conventional coagulation tests have limitations as these are focused primarily on the procoagulant factors and do not inform about platelet function and the levels/activity of von Willebrand factor, natural anticoagulants, and fibrinolytic activity.[44–47] In this scheme, viscoelastic coagulation tests can provide a panoramic assessment of the entire coagulation process from the formation to degradation of clots, platelet function, and fibrinolysis.[48] In this issue, Guaragni and Motta[49] have provided a comprehensive review of the potential benefits of viscoelastic tests in neonatal care; these tests can help identify premature/critically ill infants at higher risk of hemorrhage during routine care or after surgery and may need corrective transfusions with appropriate blood products.

Finally, this issue brings a detailed, updated review of holoprosencephaly (HPE).[50] As we know, HPE is a complex malformation reflecting failed or incomplete cleavage of the forebrain (prosencephalon) into right and left hemispheres, deep brain structures, and the olfactory and optic bulbs during the embryonic period.[51] At birth, the prevalence of HPE is 1 in 8,000–10,000 live births and stillbirths.[52–55] The etiopathogenesis is unclear, although both syndromic and isolated HPE can be heritable.[56] Syndromic HPE may involve multiple systems, including the central nervous system, eyes, hearing, olfactory, the gastrointestinal system, and the genital tracts.[51] No specific treatment is known.[57] Careful clinical and genetic evaluation is necessary for symptomatic management and for counseling families.[58]

References

1. World-Health-Organization. Newborn mortality 2024 [Available from: https://www.who.int/news-room/fact-sheets/detail/newborn-mortality.
2. Barfield WD. Standard terminology for fetal, infant, and perinatal deaths. Pediatrics. 2016;137(5):e20160551. doi: 10.1542/peds.2016-0551.
3. Mengistu TS, Schreiber V, Flatley C, Fox J, Kumar S. Factors associated with increased risk of early severe neonatal morbidity in late preterm and early term infants. J Clin Med. 2021;10(6). doi: 10.3390/jcm10061319.
4. Global-newborn-society. Every baby counts: each time we lose an infant, we lose an entire life and its potential 2024 [Available from: https://www.globalnewbornsociety.org/.
5. Barbosa VM. Teamwork in the neonatal intensive care unit. Phys Occup Ther Pediatr. 2013;33(1):5–26. doi: 10.3109/01942638.2012.729556.
6. Office-of-Disease-Prevention-and-Health-Promotion. Infants 2024 [Available from: https://health.gov/healthypeople/objectives-and-data/browse-objectives/infants.
7. National-Center-for-Health-Statistics. Births and Natality 2024 [Available from: https://www.cdc.gov/nchs/fastats/births.htm.
8. Doody O, Bailey ME. Setting a research question, aim and objective. Nurse Res. 2016;23(4):19–23. doi: 10.7748/nr.23.4.19.s5.
9. Hurn J, Kneebone I, Cropley M. Goal setting as an outcome measure: A systematic review. Clin Rehabil. 2006;20(9):756–772. doi: 10.1177/0269215506070793.
10. Madewell ZJ, Whitney CG, Velaphi S, Mutevedzi P, Mahtab S, Madhi SA, et al. Prioritizing Health Care Strategies to Reduce Childhood Mortality. JAMA Netw Open. 2022;5(10):e2237689. doi: 10.1001/jamanetworkopen.2022.37689.

11. Chen A, Oster E, Williams H. Why is infant mortality higher in the United States than in Europe? Am Econ J Econ Policy. 2016;8(2):89–124. doi: 10.1257/pol.20140224.
12. Eckhoff DO, Weiss J. Goal setting: A concept analysis. Nurs Forum. 2020;55(2):275–281. doi: 10.1111/nuf.12426.
13. Bell EA, Rufrano GA, Traylor AM, Ohning BL, Salas E. Enhancing team success in the neonatal intensive care unit: challenges and opportunities for fluid teams. Front Psychol. 2023;14:1284606. doi: 10.3389/fpsyg.2023.1284606.
14. Morley L, Cashell A. Collaboration in Health Care. J Med Imaging Radiat Sci. 2017;48(2):207–216. doi: 10.1016/j.jmir.2017.02.071.
15. Bedwell WL. Adaptive Team Performance: The influence of membership fluidity on shared team cognition. Front Psychol. 2019;10:2266. doi: 10.3389/fpsyg.2019.02266.
16. Alderwick H, Hutchings A, Briggs A, Mays N. The impacts of collaboration between local health care and non-health care organizations and factors shaping how they work: a systematic review of reviews. BMC Public Health. 2021;21(1):753. doi: 10.1186/s12889-021-10630-1.
17. Pina IL, Cohen PD, Larson DB, Marion LN, Sills MR, Solberg LI, et al. A framework for describing health care delivery organizations and systems. Am J Public Health. 2015;105(4):670–679. doi: 10.2105/AJPH.2014.301926.
18. World-Health-Organization. WHO global strategy on people-centred and integrated health services 2024 [Available from: https://www.afro.who.int/sites/default/files/2017-07/who-global-strategy-on-pcihs-main-document_final.pdf.
19. Lawn JE, Ehret DEY, Mutema T, Stevenson A, Walker K, Storari L, et al. Every Newborn Counts, Everywhere: Statement from the 2023 Joint European Neonatal Societies' (jENS) Congress. newborn (Clarksville). 2024;3(1):1–2. doi: 10.5005/jp-journals-11002-0087.
20. United-Nations-Childrens-Emergency-Fund. A Neglected Tragedy: The global burden of stillbirths 2024 [Available from: https://data.unicef.org/resources/a-neglected-tragedy-stillbirth-estimates-report/.
21. United-Nations-Department-of-Economic-and-Social-Affairs. Sustainable Development: The 17 Goals 2024 [Available from: https://sdgs.un.org/goals.
22. United-Nations-Children's-Emergency-Fund. Child survival and the SDGs 2024 [Available from: https://data.unicef.org/topic/child-survival/child-survival-sdgs/.
23. United-Nations. World Down Syndrome Day 21 March 2024 [Available from: https://www.un.org/en/observances/down-syndrome-day.
24. Down-Syndrome-International. Lots of Socks campaign 2024 [Available from: https://www.worlddownsyndromeday.org/lots-of-socks-campaign.
25. Schneider DJ. The patent ductus arteriosus in term infants, children, and adults. Semin Perinatol. 2012;36(2):146–153. doi: 10.1053/j.semperi.2011.09.025.
26. Antonarakis SE, Skotko BG, Rafii MS, Strydom A, Pape SE, Bianchi DW, et al. Down syndrome. Nat Rev Dis Primers. 2020;6(1):9. doi: 10.1038/s41572-019-0143-7.
27. Graupe MH, Naylor CS, Greene NH, Carlson DE, Platt L. Trisomy 21. Second-trimester ultrasound. Clin Perinatol. 2001;28(2):303–319. doi: 10.1016/s0095-5108(05)70085-3.
28. Devlin L, Morrison PJ. Accuracy of the clinical diagnosis of Down syndrome. Ulster Med J. 2004;73(1):4–12. PMID: 15244118.
29. Berisha SZ, Shetty S, Prior TW, Mitchell AL. Cytogenetic and molecular diagnostic testing associated with prenatal and postnatal birth defects. Birth Defects Res. 2020;112(4):293–306. doi: 10.1002/bdr2.1648.
30. Biesecker LG, Spinner NB. A genomic view of mosaicism and human disease. Nat Rev Genet. 2013;14(5):307–320. doi: 10.1038/nrg3424.
31. Papavassiliou P, York TP, Gursoy N, Hill G, Nicely LV, Sundaram U, et al. The phenotype of persons having mosaicism for trisomy 21/Down syndrome reflects the percentage of trisomic cells present in different tissues. Am J Med Genet A. 2009;149A(4):573–583. doi: 10.1002/ajmg.a.32729.
32. Orr B, Godek KM, Compton D. Aneuploidy. Curr Biol. 2015;25(13):R538–42. doi: 10.1016/j.cub.2015.05.010.
33. Mohan S, Badami K, Kumar P, Shilpa YD, Hemalata BC, Tumbadi K. Prediction of retinopathy of prematurity in single and twin babies: The predictive accuracy of WINROP. newborn (Clarksville). 2024;3(1):3–7. doi: 10.5005/jp-journals-11002-0084.
34. Sahota R, Singh V, Parveen H, Kaur N, Upreti N, Mahindre A, et al. Comparison of transcutaneous bilirubin with total serum bilirubin levels before, during, and post-phototherapy in preterm and term newborns in Uttarakhand, India. newborn (Clarksville). 2024;3(1):8–12. doi: 10.5005/jp-journals-11002-0086.
35. Hameed NN, Khaleel MM, Saugstad OD. Timely respiratory support can improve clinical outcomes of premature infants in a country with limited medical resources due to chronic conflicts. newborn (Clarksville). 2024;3(1):13–18. doi: 10.5005/jp-journals-11002-0088.
36. Guay JM, Carvi D, Raines DA, Luce WA. Care of the neonate on nasal continuous positive airway pressure: A Bedside Guide. Neonatal Netw. 2018;37(1):24–32. doi: 10.1891/0730-0832.37.1.24.
37. Maheshwari A, Mantry H, Bagga N, Frydrysiak-Brzozowska A, Badarch J, Rahman MM. Milk Fat Globules: 2024 Updates. newborn (Clarksville). 2024;3:19–37. doi. 10.5005/jp-journals-11002-0085.
38. Maheshwari A. Fats in Human Milk: 2022 Updates on Chemical Composition. newborn (Clarksville). 2022;1(4):384–396. doi: 10.5005/jp-journals-11002-0050.
39. Ross MG, Kavasery MP, Cervantes MK, Han G, Horta B, Coca KP, et al. High-fat, high-calorie breast milk in women with overweight or obesity and its association with maternal serum insulin concentration and triglycerides levels. Children (Basel). 2024;11(2). doi: 10.3390/children11020141.
40. Thum C, Roy NC, Everett DW, McNabb WC. Variation in milk fat globule size and composition: A source of bioactives for human health. Crit Rev Food Sci Nutr. 2023;63(1):87–113. doi: 10.1080/10408398.2021.1944049.
41. Manoni M, Di Lorenzo C, Ottoboni M, Tretola M, Pinotti L. Comparative proteomics of milk fat globule membrane (mfgm) proteome across species and lactation stages and the potentials of mfgm fractions in infant formula preparation. Foods. 2020;9(9). doi: 10.3390/foods9091251.
42. Raza GS, Herzig KH, Leppaluoto J. Invited review: Milk fat globule membrane–A possible panacea for neurodevelopment, infections, cardiometabolic diseases, and frailty. J Dairy Sci. 2021;104(7):7345–7363. doi: 10.3168/jds.2020-19649.
43. Davenport P, Sola-Visner M. Hemostatic challenges in neonates. Front Pediatr. 2021;9:627715. doi: 10.3389/fped.2021.627715.
44. Palta S, Saroa R, Palta A. Overview of the coagulation system. Indian J Anaesth. 2014;58(5):515–523. doi: 10.4103/0019-5049.144643.
45. Mehic D, Colling M, Pabinger I, Gebhart J. Natural anticoagulants: A missing link in mild to moderate bleeding tendencies. Haemophilia. 2021;27(5):701–709. doi: 10.1111/hae.14356.

46. Strauss T, Elisha N, Ravid B, Rosenberg N, Lubetsky A, Levy-Mendelovich S, et al. Activity of Von Willebrand factor and levels of VWF-cleaving protease (ADAMTS13) in preterm and full term neonates. Blood Cells Mol Dis. 2017;67:14–17. doi: 10.1016/j.bcmd.2016.12.013.
47. Buyukinan M, Yilmaz D, Yalaz M, Koroglu OA, Akisu M, Kavakli K, et al. Long-term ongoing coagulopathy in premature infants with respiratory distress syndrome. Clin Appl Thromb Hemost. 2013;19(5):535–540. doi: 10.1177/1076029612444003.
48. Hartmann J, Hermelin D, Levy JH. Viscoelastic testing: an illustrated review of technology and clinical applications. Res Pract Thromb Haemost. 2023;7(1):100031. doi: 10.1016/j.rpth.2022.100031.
49. Guaragni B, Motta M. Hemostasis assessment in neonates: Evaluation of viscoelastic properties of blood clots. newborn (Clarksville). 2024;3(1):38–44. doi: 10.5005/jp-journals-11002-0089.
50. Maheshwari A, Huisman TAGM. Holoprosencephaly. newborn (Clarksville). 2024;3(1):45–60. doi: 10.5005/jp-journals-11002-0083.
51. Dubourg C, Bendavid C, Pasquier L, Henry C, Odent S, David V. Holoprosencephaly. Orphanet J Rare Dis. 2007;2:8. doi: 10.1186/1750-1172-2-8.
52. Raam MS, Solomon BD, Muenke M. Holoprosencephaly: a guide to diagnosis and clinical management. Indian Pediatr. 2011;48(6):457–466. doi: 10.1007/s13312-011-0078-x.
53. Bullen PJ, Rankin JM, Robson SC. Investigation of the epidemiology and prenatal diagnosis of holoprosencephaly in the North of England. Am J Obstet Gynecol. 2001;184(6):1256–1262. doi: 10.1067/mob.2001.111071.
54. Yi L, Liu Z, Deng C, Li X, Wang K, Deng K, et al. Epidemiological characteristics of holoprosencephaly in China, 2007-2014: A retrospective study based on the national birth defects surveillance system. PLoS One. 2019;14(6):e0217835. doi: 10.1371/journal.pone.0217835.
55. Malta M, AlMutiri R, Martin CS, Srour M. Holoprosencephaly: Review of Embryology, Clinical Phenotypes, Etiology and Management. Children (Basel). 2023;10(4). doi: 10.3390/children10040647.
56. Geng X, Oliver G. Pathogenesis of holoprosencephaly. J Clin Invest. 2009;119(6):1403–1413. doi: 10.1172/JCI38937.
57. Kauvar EF, Muenke M. Holoprosencephaly: recommendations for diagnosis and management. Curr Opin Pediatr. 2010;22(6):687–695. doi: 10.1097/MOP.0b013e32833f56d5.
58. Hadley DW, Kruszka P, Muenke M. Challenging issues arising in counseling families experiencing holoprosencephaly. Am J Med Genet C Semin Med Genet. 2018;178(2):238–245. doi: 10.1002/ajmg.c.31627.

Akhil Maheshwari, MD
Kei Lui, MD
Mario Motta, MD

COMMENTARY

Every Newborn Counts, Everywhere: Statement from the 2023 Joint European Neonatal Societies' (jENS) Congress

Joy E Lawn[1,2], Danielle EY Ehret[3,4], Tendai Mutema[5], Alex Stevenson[6], Karen Walker[7,8], Linda Storari[9], Willem P de Boode[10], Silke Mader[11], Corrado Moretti[12], Charles C Roehr[13,14], Ola D Saugstad[15,16]

Received on: 28 January 2024; *Accepted on:* 29 February 2024; *Published on:* 26 March 2024

Keywords: Baby, Global health, Infant, Low- and middle-income countries, Newborn, Neonate.
Newborn (2024): 10.5005/jp-journals-11002-0087

Introduction

Every newborn enters the world the same, but where you are born determines your access to care and your survival. Annually, 2.3 million newborns die, in addition to 1.9 million stillbirths, with 98% in low- and middle-income countries, and only 0.6% in Europe. Almost all these deaths are preventable. Collectively, we can prevent millions of newborn deaths and the majority of stillbirths within a generation.[1] There are totally 134 million births annually, and Table 1 presents the number (%) of births per continent.[2]

Neonatal conditions are the leading cause of child deaths and of lost human capital through the whole life course, in the Global Burden of Disease, number one since 1990. Conflicts, climate change, COVID-19 and the cost of living crisis are all impacting healthcare systems, but newborns are the most vulnerable.[3]

Progress to reduce neonatal mortality is unacceptably slow, especially in countries with the highest burden, and 63 countries are off track for their newborn targets by 2030 in the sustainable development goals (SDGs).[4] Highly effective interventions exist and the *Every Newborn Action Plan* lays out a strategic and cost-effective roadmap, with 106 countries committed to action. We have evidence and new opportunities to accelerate progress in all settings, but urgent action is needed.[5] The priority is higher coverage to reach every country and every district with maternal and neonatal care. To reach the SDG target of <12 neonatal deaths per 1000 live births requires high coverage of respiratory support for preterm newborns. The following actions are the foundation.[3]

Invest

It is not just more money, but smarter and fairer investment that is required. There are $15.9 billion a year of donor aid to spend on Reproductive, Maternal, Newborn, and Child Health (RMNCH) yet, newborns, and especially stillbirths, are still rarely mentioned.

Table 1: Annual newborn births by continent

Oceania	0.69	(0.5)
Latin America and the Caribbean	9.71	(7.2)
North America	4.10	(3.1)
Europe	6.88	(5.1)
Africa	45.63	(34.0)
Asia	67.23	(50.1)

Number in million (percentage) of births per continent (2021). Data from reference 2

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom
[2]NEST360 Alliance
[3]University of Vermont Larner College of Medicine, Burlington, Vermont, United States of America
[4]Vermont Oxford Network, Burlington, Vermont, United States of America
[5]Family Lead African Neonatal Network
[6]African Neonatal Association, Kigali, Rwanda
[7]Department of Newborn Care, Royal Prince Alfred Hospital; Faculty of Medicine and Health, University of Sydney, Sydney, Australia
[8]Council of International Neonatal Nurses Inc.
[9]Global Health, Chiesi Farmaceutici SpA, Parma, Italy
[10]Department of Neonatology, Radboud University Medical Center, Radboud Institute for Health Sciences, Amalia Children's Hospital, Nijmegen, the Netherlands
[11]European Foundation for the Care of Newborn Infants, Munich, Germany
[12]Department of Paediatrics, Sapienza University of Rome, Italy
[13]National Perinatal Epidemiology Unit, Oxford Population Health, University of Oxford, Oxford, United Kingdom; and Faculty of Health Sciences, University of Bristol, Bristol, United Kingdom
[14]Faculty of Health Sciences, University of Bristol, Bristol, United Kingdom
[15]Department of Pediatric Research, University of Oslo, Norway
[16]Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States of America

Corresponding Author: Ola D Saugstad, Department of Pediatric Research, University of Oslo, Norway; Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States of America, Phone: +4790194144, e-mail: o.d.saugstad@medisin.uio.no

How to cite this article: Lawn JE, Ehret DEY, Mutema T, *et al*. Every Newborn Counts, Everywhere: Statement from the 2023 Joint European Neonatal Societies' (jENS) Congress. Newborn 2024;3(1): 1–2.

Source of support: Nil

Conflict of interest: None

Furthermore, the return on investment is very high. Recent analyses from Tanzania shows a return of $8–12 for every $1 invested in neonatal care improvements.[6]

Implement

Sustainable systems change is required at all levels of health care from facilities to national and subnational policies and programs, including the right data and right healthcare professionals to track and drive faster progress.

Integrate

Collaborate across national and professional boundaries. Support local networks and learning communities. Accountability, based on data is key.

Innovate

Ensure innovations are affordable and available and used in the highest burden countries.[7,8]

All of us have a role to play to raise the urgency of this silent public health and human rights epidemic. Family's voices must be amplified and acted upon. We can and must partner with communities, healthcare professionals and leaders to change life chances for the next generation everywhere. Each time we lose an newborn, we lose an entire life and its possibilities!

Authors' Contributions

AS, DE, JEL, KW, ODS, and TM wrote the first drafts. All authors have commented on and approved the final version. CCR and WdeB are past and present presidents of the European Society for Paediatric Research. CM is president of the Union of European Perinatal and Neonatal Societies. SM is chairperson of the European Foundation for the Care of Newborn Infants. AS is president of the African Neonatal Association. KW is president of the Council of International Neonatal Nurses.

References

1. Lawn JE, Bhutta ZA, Ezeaka C, et al. Ending preventable neonatal deaths: Multicountry evidence to inform accelerated progress to the sustainable development goal by 2030. Neonatology 2023;120(4):491–499. DOI: 10.1159/000530496.
2. https://ourworldindata.org/grapher/annual-number-of-births-by-world-region.
3. WHO. Born too soon: Decade of action on preterm birth. WHO, Geneva; 2023. Available from: https://www.who.int/publications/i/item/9789240073890.
4. WHO. Improving maternal and newborn health and survival and reducing stillbirth—Progress report 2023. WHO, Geneva; 2023. Available from: https://www.who.int/publications/i/item/9789240073678.
5. Kumar MB, Bath D, Binyaruka P, et al. Donor aid mentioning newborns and stillbirths, 2002–19: An analysis of levels, trends, and equity. The Lancet Global Health 2023;11(11):e1785–e1793. DOI: 10.1016/S2214-109X(23)00378-9.
6. Kamuyu R, Tarus A, Bundala F, et al. Investment case for small and sick newborn care in Tanzania: Systematic analyses. BMC Pediatr 2023;23(Suppl 2):632. DOI: 10.1186/s12887-023-04414-2.
7. Cutler DM, Meara E, Richards-Shubik S. Induced Innovation and Social Inequality: Evidence from Infant Medical Care. J Hum Resour 2012;47(2):456–492. DOI: 10.3368/jhr.47.2.456.
8. Burstein R, Henry NJ, Collison ML, et al. Mapping 123 million neonatal, infant and child deaths between 2000 and 2017. Nature 2019;574:353–358. DOI: 10.1038/s41586-019-1545-0.

ORIGINAL RESEARCH

Prediction of Retinopathy of Prematurity in Single and Twin Babies: The Predictive Accuracy of WINROP

S Mohan[1], Kalpana Badami[1], Pavan Kuman[1], Shilpa YD[1], Hemalata BC[1], Kavitha Tumbadi[1]

Received on: 22 December 2023; *Accepted on*: 23 January 2024; *Published on*: 26 March 2024

Abstract

Aim: To test the effectiveness of WINROP software tool to screen retinopathy of prematurity (ROP) in Indian preterm infant population including twin neonates.

Materials and methods: In a retrospective single-center study, birth weight (BW), gestational age (GA), comorbidities, and weekly weight measurements (for 5 weeks) were retrieved from 63 preterm infants born between 01/2014 and 04/2015. The obtained data were entered into the WINROP algorithm to obtain ROP outcomes and WINROP alarm.

Results: For a cohort of 63 patients together with twin neonates, the median BW was 1250 gm and GA was 30 weeks. Of the 63 infants, 22 infants developed type I ROP and 39 infants developed type II ROP. WINROP alarm was triggered in 33 (52.38%) infants. Comorbidities, such as malnutrition, respiratory distress syndrome (RDS), blood transfusion, anemia of prematurity, and pregnancy-induced hypertension (PIH) were associated with the development of ROP. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of WINROP to predict type I ROP were 63.6, 53.6, 42.4, and 73.3%, respectively. In twin neonates, WINROP predicted type I ROP with sensitivity, specificity, PPV, and NPV of 100, 60, 33.3, and 100%, respectively.

Conclusion: This is the first WINROP validation study in twin neonates from Indian settings. The WINROP model was highly sensitive to detect type I ROP in twin neonates. However, due to low specificity and low PPV, the outcome of this study suggests the use of WINROP algorithm alongside standard ROP screening in infants including twin neonates with WINROP alarm.

Keywords: India, Retinopathy of prematurity, Twins, Type I ROP, WINROP.

Newborn (2024): 10.5005/jp-journals-11002-0084

Introduction

Retinopathy of prematurity (ROP), a gestational age (GA)-related illness, is a leading cause of childhood blindness in premature infants.[1] Pathologically, ROP involves delayed retinal vascularization, vaso-proliferation, and intravitreal angiogenesis.[2] Fibrovascular retinal detachment and permanent blindness are the risks associated with ROP.[2] Among the risk factors, low GA, low birth weight (BW), and oxygen level are the major risk factors for the development of ROP.[1] Timely screening of premature infants for ROP could improve visual prognosis. The conventional ROP screening involves dilation of pupil and subsequent use of indirect ophthalmoscopy and retinal imaging using a RetCam.[3] However, to minimize the risk and to increase the identification of high-risk infants, researchers have introduced and developed multiple prediction models, such as WINROP, CO-ROP, ROP Score, and CHOP-ROP.[1] Several studies have show that the WINROP model may have sensitivity levels as high as 80–90% in preterm infants.[4–6]

Since, low GA and low BW are the major risk factors in the development of ROP, WINROP prediction model uses weight and GA at birth as a dichotomized factor for the screening of ROP.[7] However, studies indicate that heavier and more mature babies can also develop ROP, especially, from middle-income or developing countries such as India indicating the role of alternative risk factors in the development of ROP.[1] Thus, to accommodate larger number of ROP screening, the National guidelines for the screening for ROP has broad eligibility criteria with GA of ≤34 weeks or <2000 gm or neonates with GA above 34 weeks and associated risk factors, such as prolonged oxygen support, cardiovascular instability, and sepsis.[3]

[1]Department of Vitreo-Retina, Minto Ophthalmic Hospital, Bengaluru, Karnataka, India

Corresponding Author: S Mohan, Department of Vitreo-Retina, Minto Ophthalmic Hospital, Bengaluru, Karnataka, India, Phone: +91 7406413111, e-mail: smohanms17224w@gmail.com

How to cite this article: Mohan S, Badami K, Kumar P, *et al*. Prediction of Retinopathy of Prematurity in Single and Twin Babies: The Predictive Accuracy of WINROP. Newborn 2024;3(1):3–7.

Source of support: Nil

Conflict of interest: None

In the last 5 years, several clinical studies performed in India have adopted the WINROP model[8,9] and have shown high sensitivity and NPV of the algorithm to predict type I ROP. The model was sensitive to predict type I ROP earliest at 2 weeks than the conventional screening which predicted ROP at 7th week postnatal stage.[9] However, there is scarcity of relevant-studies and the existing literature does not completely validate the adoption of WINROP in Indian settings.

Aim and Objectives

The aim of the present study was to test the efficacy of WINROP to predict ROP in Indian preterm infant population. The objective was (i) to investigate the pattern of ROP in preterm infant population including twin neonates and (ii) to measure the diagnostic performance of WINROP software to predict ROP in twin neonates.

Materials and Methods

This was a retrospective study conducted on 63 infants born between 01/2014 and 04/2015 and who were at high risk of developing ROP. Infants with the following criteria were included: (i) BW less than 1500 gms; (ii) GA 30 weeks; (iii) infants with BW between 1500 gm and 2000 gm or (iv) GA more than 30 weeks and with an unstable clinical course and at high risk for ROP. The enrolled infant population also included 6 pairs (12 infants) of twin neonates with ROP. Twins were called discordant if their BW difference was more than 15%. For twins, the inclusion criteria were that both twin infants were alive. Infants with congestive heart failure, neonatal nephrotic syndrome, hydrocephalus were excluded due to nonphysiologically weight gain. Data of final ROP outcome should be available.

WINROP Screening

For WINROP screening, following clinical data were retrospectively retrieved including infant's GA (less than 32 weeks at birth), BW, associated comorbidity, weekly weight measurements, physiological weight gain and absence of other pathologic retinal vascular disease. The collected data were entered into WINROP software (https://winrop.com/). Clinical examination for ROP was performed weekly/twice in a week and close observation was made to see the progression of ROP to type I ROP. The clinical phenotypes of ROP, namely, type I and type II ROP were classified as per the Early Treatment of Retinopathy of Prematurity (ETROP) cooperative group classification.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 24 was used for descriptive statistics. Association between the variables was estimated by Chi-square analysis. Independent *t*-test was performed to evaluate the differences in variables between with and without WINROP group. The sensitivity, specificity, PPVs, and NPVs of the WINROP algorithm were also calculated.

Results

Table 1 illustrates the demographic characteristics and ROP outcomes in 63 preterm infants who were enrolled in this study. The majority were female infants (53.9%) with very low BW in the range of 1000–1500 gm (69.8%). The median GA was 30.0 weeks (30.0–32.0 weeks) and the median BW was 1250 gm (range: 1052.5–1500.0 gm). The median BW in the first week was 1310 gm (1100.0–1547.50 gm) which increased to 1587 gm (range: 1353.75–1797.5 gm) in the fifth week. Based on birth plurality, out of 63 preterm infants, 51 were single babies and 12 (6 pairs) were twin babies. WINROP alarm was signaled in 52.3% of infants and 39 (61.9%) infants developed type II ROP, 22 (34.9%) developed type I ROP and 2 (3.1%) had no ROP.

Table 1: Demographic characteristics

	Frequency (%)
Birth weight	
ELBW	5 (7.94)
LBW	14 (22.22)
VLBW	44 (69.84)
Gender	
Male	29 (46.03)
Female	34 (53.97)
WINROP	
Alarm	33 (52.38)
No alarm	30 (47.62)
Birth plurality	
Single	51 (80.95)
Twins	12 (19.05)
Type of ROP	
No ROP	2 (3.17)
Type II ROP	39 (61.9)
Type I ROP	22 (34.92)
Median (IQR)	
Gestational age (weeks)	30.000 (30.000–32.000)
Birth weight (BW in gm)	1250.00 (1052.50–1500.00)
Weight in week 1	1310.00 (1100.00–1547.50)
Weight in week 2	1365.000 (1157.50–1608.75)
Weight in week 3	1440.00 (1203.75–1682.5)
Weight in week 4	1495.00 (1263.75–1732.5)
Weight in week 5	1587.50 (1353.75–1797.5)

Association between Birth Characteristics and WINROP

Table 2 presents the association of birth characteristics and type of ROP with and without WINROP alarm. There was no association of WINROP alarm with sex ($\chi^2 = 0.009$, $p > 0.05$), BW ($\chi^2 = 3.171$, $p > 0.05$) and type of ROP ($\chi^2 = 3.527$, $p > 0.05$). Further, GA ($t = -1.238$, $p > 0.05$), BW ($t = -1.788$, $p > 0.05$) and physiological weight gain from 1st week to 5th week were not associated with WINROP alarm ($p > 0.05$).

Association between Type of ROP and Comorbidities

Infants had multiple comorbidities including RDS (76.1%), followed by blood transfusion (44.4%) anemia of prematurity (42.8%), hyaline membrane disease (23.8%) and malnutrition (22.2%). In addition, about 76.1% of infants were born to mother with PIH. ROP showed highly significantly association with malnutrition ($\chi^2 = 20.46$, $p < 0.001$), RDS ($\chi^2 = 9.33$, $p < 0.001$) and anemia of prematurity ($\chi^2 = 9.58$, $p < 0.001$) and significant association with blood transfusion ($\chi^2 = 6.48$, $p < 0.05$) and PIH ($\chi^2 = 7.28$, $p < 0.05$). Type I ROP was associated with malnutrition and anemia of prematurity and type II ROP was associated with RDS, blood transfusion and PIH.

Effectiveness of WINROP to Predict ROP

WINROP alarm was signaled in 33 infants, out of which 14 (42.4%) developed type I ROP and 19 (57.6%) developed non-type I ROP. About 8 infants developed type I ROP without any WINROP alarm. In the prediction of type I ROP, WINROP tool had a sensitivity of 63.6%, specificity of 53.6%, PPV of 42.4% and the NPV of 73.3% (Table 3).

WINROP Analysis for Twin Neonates

Table 4 presents the association of birth characteristics and type of ROP with and without WINROP alarm in twin neonates. Sex and type of ROP were not associated with WINROP alarm. The association of WINROP alarm with BW was significant ($\chi^2 = 4.00$, $p < 0.05$). WINROP alarm was signaled in twins with very low BW suggestive of high risk of ROP in infants with very low BW (1000–1500 gm). Further, the *t*-test showed significant difference in BW between infants with and without WINROP alarm ($t = -2.533$, $p < 0.05$) (Table 4).

Table 2: Association of birth characteristics and type of ROP with WINROP

	WINROP		*Chi-square/ t-value*	*p-value*
	Alarm	*No alarm*		
Sex				
Male	15 (51.7%)	14 (48.3%)	0.009	0.923
Female	18 (52.9%)	16 (47.1%)		
Birth weight				
ELBW	4 (80%)	1 (20%)	3.171	0.205
LBW	5 (35.7%)	9 (64.3%)		
VLBW	24 (54.5%)	20 (45.5%)		
Type of ROP				
No ROP	0 (0%)	2 (100%)	3.527	0.171
Type II ROP	19 (48.7%)	20 (51.3%)		
Type I ROP	14 (63.6%)	8 (36.4%)		
Birth weight	1197.72 ± 292.73	1325.33 ± 271.54	–1.788	0.079
Gestational age	31.0 ± 1.73	31.60 ± 2.11	–1.238	0.221
Weight in 1st week	1275.15 ± 305.78	1377.32 ± 272.82	–1.357	0.180
Weight in 2nd week	1324.39 ± 302.93	1433.92 ± 273.08	–1.472	0.146
Weight in 3rd week	1388.42 ± 301.96	1498.21 ± 276.06	–1.471	0.146
Weight in 4th week	1442.87 ± 312.96	1555.89 ± 277.51	–1.480	0.144
Weight in 5th week	1515.42 ± 308.59	1628.57 ± 276.70	–1.496	0.140

Table 3: Sensitivity, specificity, PPV, and NPV in predicting type I ROP using the WINROP

	ROP			*Sensitivity (%)*	*Specificity (%)*
	Type I ROP	*Non-type I ROP*	*Total*		
WINROP					
Alarm	14	19	33	63.64	53.66
No alarm	8	22	30		
Predictive value (%)					
PPV	42.4				
NPV	73.3				

NPV, negative predictive value; PPV, positive predictive value

Table 4: Association between birth characteristics of twins and type of ROP with WINROP

	WINROP		*Chi-square/ t-value*	*p-value*
	Alarm	*No alarm*		
Sex				
Male	0 (0%)	2 (100%)	2.400	0.121
Female	6 (60%)	4 (40%)		
Birth weight				
LBW	0 (0%)	3 (100%)	4.000	0.046
VLBW	6 (66.7%)	3 (33.3%)		
Type of ROP				
No ROP	0 (0%)	2 (100%)	4.000	0.135
Type II ROP	4 (50%)	4 (50%)		
Type I ROP	2 (100%)	0 (0%)		
Gestational age (GA)	30.6 ± 2.0	33 ± 2.4	–1.784	0.105
Birth weight (BW)	1092.5 ± 90.76	1410 ± 293.25	–2.533	0.030
Weight in week 1	1170.0 ± 85.3	1397.5 ± 272.4	–1.788	0.117
Weight in week 2	1201.6 ± 99.2	1455.0 ± 266.6	–2.166	0.062
Weight in week 3	1270.8 ± 95.8	1522.5 ± 276.9	–2.099	0.069
Weight in week 4	1323.3 ± 105.8	1583.7 ± 265.1	–2.209	0.058
Weight in week 5	1390.8 ± 101.3	1653.7 ± 270.0	–2.217	0.057

The mean value of BW was lower in WINROP alarm group than no alarm group (1092.5 ± 90.7 vs 1410 ± 293.2 gm).

In 6 pairs (12 babies) of twin babies, RDS (83.3%), anemia of prematurity (75%), and hyaline membrane disease (50%) were the most frequent comorbidities. Among the comorbidities in twin babies, malnutrition ($\chi^2 = 12.00$, $p < 0.001$) and PIH ($\chi^2 = 7.33$, $p < 0.05$) showed significant association with type I ROP, and RDS ($\chi^2 = 12.00$, $p < 0.001$) and anemia of prematurity ($\chi^2 = 7.33$, $p < 0.05$) showed significant association with type II ROP.

In twin neonates, WINROP alarm was signaled in six infants, out of which two developed type I ROP and six infants with no alarm developed non-type I ROP. The diagnostic performance of WINROP indicated sensitivity of 100%, specificity of 60%, PPV of 33.33%, and NPV of 100% to predict type I ROP (Table 5).

Discussion

Clinically, prediction models such as WINROP have been used to detect the risk of ROP in preterm infants.[5,10] The present retrospective study was performed to analyze the predictive ability of WINROP algorithm for the detection of ROP in preterm infant babies in Indian setup. The median BW of 1250 gm and GA of 30 weeks were comparable to studies from other Asian population including China[11] and Taiwan.[12] WINROP alarm was signaled in 52.3% of infants which was low compared with previous studies from Malaysia (72.8%),[13] India (74.2%),[8] and Saudi (70.9%)[14] but

Table 5: Sensitivity, specificity, PPV, and NPV in predicting type I ROP using the WINROP

	ROP			Sensitivity	Specificity
	Type I ROP	*Non-type I ROP*	*Total*	*(%)*	*(%)*
WINROP					
Alarm	2	4	6	100	60
No alarm	0	6	6		
Predictive value (%)					
PPV	33.33				
NPV	100				

higher than percent of WINROP alarm in the study from Australia (42.6%)[6] and another study from India (27.7%).[15] In the present study, WINROP alarm showed no association with BW ($p > 0.05$), sex ($p > 0.05$) and type of ROP ($p > 0.05$). Contrary to the present finding Sute et al.[15] showed association of WINROP alarm with gestation age, BW and type I ROP. Previous studies have associated multiple comorbidities, such as RDS, blood transfusion, dysplasia, large patent ductus arteriosus and septic shock have been associated with ROP;[13,15,16] however, in this study, malnutrition ($p < 0.001$), RDS ($p < 0.001$), anemia of prematurity ($p < 0.01$), blood transfusion ($p < 0.05$) and PIH ($p < 0.05$) were associated with ROP. Across different studies,[15,16] RDS was the most common comorbidity which was associated with ROP. Studies indicate that disruption of gas exchange leads to multiple complications including increased risk of ROP in preterm infants.[17] Studies state that parenteral nutrition consisting of high energy and protein, and mother's milk to ensure optimal growth and adiposity in the postnatal weeks may have a protective effect against the development of ROP in preterm infants.[18,19] Likewise, anemia and blood transfusion has been reported as an independent risk factor of ROP in premature infants.[20] On the contrary, in the literature, the effect of maternal PIH on the occurrence of ROP is not conclusive and requires further analysis.[21] Overall, based on the present findings, it can be inferred that multiple neonatal risk factors are associated with the development of ROP and effort should be made to control these factors to reduce the risk of ROP.

In various cohorts, the sensitivity and specificity of WINROP to predict ROP has varied. In the present study, the sensitivity to predict type I ROP through WINROP was low (63.6%) compared with previous studies from countries, such as Saudi (100%),[14] Sweden (100%),[22] and Malaysia (95.2%)[13] which reported high sensitivity but comparable to previous studies from Taiwan (64.7%)[10] and South Africa (72.9%).[5] Further, the specificity of WINROP was low (53.6%) but comparable to previous studies in the literature from Malaysia (33.8%)[13] and Japan (42.7%).[23] A very few Indian studies have used the WINROP algorithm for the prediction of ROP in preterm infants.[8,15] The use of the WINROP model in Indian cohorts has reported sensitivity of 90.3%[8] and 80%,[15] and specificity of 38.4%[8] and 80.6%.[15] Further, the other validation parameter, such as PPV of 42.4% and NPV of 73.3% was comparatively lower with the values mentioned in the Indian setup. In our study, 42% (14/33) of infants with WINROP alarm developed type I ROP and remaining 57% non-type I ROP suggestive of timely testing for the progression of ROP. Based on the given validation parameters of WINROP algorithm, it can be inferred that in infants with WINROP alarm, there is a requirement of ROP screening, and this could reduce the number of unnecessary screenings in preterm infants.

The present study is the inclusion of twin neonates and this is the first study from Indian babies using the WINROP model to predict ROP in twin neonates. Multiple studies have reported association of multiple gestations/twins or multiplets with the risk of ROP.[1] Of the two dichotomous variables, namely, BW and GA which is used in WINROP algorithm,[7] twin babies have the same GA and perinatal risk factors. Thus, they provide a good model to analyze if BW has a role in the progression of ROP.[24] Focused on that, the present study tested the efficacy of the WINROP model to predict ROP in twin neonates. In the present study, in twin babies, WINROP alarm was associated with very low BW ($p < 0.05$) and ROP was significantly associated with comorbidities including malnutrition ($p < 0.001$), RDS ($p < 0.001$), anemia ($p < 0.05$) and PIH ($p < 0.05$); however, further research is required to ascertain the associated factors in twin neonates with ROP. Previous studies have reported higher risk of ROP in discordant twins with lower BWs;[25] however, Azad et al.[24] states that the BW as a factor to screen ROP in twins should be performed with caution. The authors state that presentation and progression of ROP can vary in twins as heavier siblings were also presented with severe ROP. On the contrary, Sanghi et al.[26] reported that birth order, BW, and post-gestational neonatal risk factors do not predict the severity of ROP in twins. Furthermore, the WINROP model predicted type I ROP in twin babies with a high sensitivity of 100% and NPV value of 100% in this study. As stated by Sanghi et al.[8] NPV of 100% presents an ideal situation which can reduce the ROP screening for infants with no alarm. In support of this, Raffa et al.[14] argues that since prediction of ROP is important to prevent blindness, sensitivity and NPV are more relevant than other parameters to screen preterm infants with ROP.

The differences in parameters from the previous studies could be multifactorial including the study design, preterm study population, types of ROP, screening criteria for ROP, provisions for perinatal and postnatal care.[14,15] For instance, studies from Indian clinical settings, such as Sute et al.[15] performed a prospective observational study on 102 singleton preterm infants, whereas our study was retrospective in nature with 63 preterm infants including 12 twin neonates; hence, discrepancies in data within the same geographical settings cannot be avoided. Nevertheless, Ko et al.[10] states that WINROP is an effective tool to predict ROP in infants that meet the criteria of BW and GA of less than 1,000 gm and less than 28 weeks, respectively. However, for infants from developing country such as Asian preterm infant population, the ROP epidemiology and weight gain curve can differ from the developed country; the development of individualized algorithm for different geographical zones is recommended.[10] Based on the present findings that WINROP alarm was signaled in non-type I ROP also, thus, it is recommended to monitor for the progression of non-type I ROP to type I ROP. In addition, considering the small size of population of twin neonates, further validation studies to precisely estimate the sensitivity and specificity of WINROP algorithm and the generalizability of findings on a larger population of twin neonate is suggested.

The study is limited by retrospective design, small sample size and from a single center. Further studies should include prospective design on a larger infant population including twin neonates and from multicenter. Additionally, to improve the predictive efficacy of the algorithm, WINROP model should be modified to accommodate populations with different characteristics such as larger and older babies, and additionally multiple postnatal risk factors should be incorporated.

Conclusion

Overall, the WINROP model had a moderate sensitivity of 63.6%, low specificity of 53.6%, low PPV of 42.4% and high NPV of 73.3% to predict type I ROP. Further, WINROP had a high sensitivity of 100%, a high NPV of 100% but low specificity of 60% and low PPV of 33% to predict type I ROP in twin neonates. Based on these performance parameters, it is suggested that WINROP algorithm be used potentially as an accessory tool and standard ROP screening be performed alongside on infants with WINROP alarm.

References

1. Kim SJ, Port AD, Swan R, et al. Retinopathy of prematurity: A review of risk factors and their clinical significance. Surv Ophthalmol [Internet]. 2018;63(5):618–637. DOI: 10.1016/j.survophthal.2018.04.002.
2. Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. Ophthalmology 2015;122(1):200–210. DOI: 10.1016/j.ophtha.2014.07.050.
3. Shukla R, Murthy GVS, Gilbert C, et al. Operational guidelines for ROP in India: A summary. Indian J Ophthalmol 2020;68(Suppl 1):S108–S114. DOI: 10.4103/ijo.IJO_1827_19.
4. Sun H, Kang W, Cheng X, et al. The use of the WINROP screening algorithm for the prediction of retinopathy of prematurity in a Chinese population. Neonatology 2013;104(2):127–132. DOI: 10.1159/000351297.
5. Kesting SJ, Nakwa FL. Prediction of retinopathy of prematurity using the winrop (weight, IGF-1, neonatal retinopathy of prematurity) algorithm in a South African Population. Front Pediatr 2022;10:812404. DOI: 10.3389/fped.2022.812404.
6. Desai S, Athikarisamy SE, Lundgren P, et al. Validation of WINROP (online prediction model) to identify severe retinopathy of prematurity (ROP) in an Australian preterm population: A retrospective study. Eye [Internet] 2021;35(5):1334–1339. DOI: 10.1038/s41433-020-1094-7.
7. Binenbaum G. Algorithms for the prediction of retinopathy of prematurity based on postnatal weight gain. Clin Perinatol 2013;40(2):261–270. DOI: 10.1016/j.clp.2013.02.004.
8. Sanghi G, Narang A, Narula S, et al. WINROP algorithm for prediction of sight threatening retinopathy of prematurity: Initial experience in Indian preterm infants. Indian J Ophthalmol 2018;66(1):110–113. DOI: 10.4103/ijo.IJO_486_17.
9. Thomas D, Madathil S, Thukral A, et al. Diagnostic accuracy of WINROP, CHOP-ROP and ROPScore in detecting type 1 retinopathy of Prematurity. Indian Pediatr 2021;58(10):915–921. DOI: 10.1007/s13312-021-2321-4.
10. Ko C, Kuo H, Chen C, et al. Using WINROP as an adjuvant screening tool for retinopathy of prematurity in southern Taiwan. Am J Perinatol 2015;30(2):149–154. DOI: 10.1055/s-0034-1376389.
11. Yau GS, Lee JW, Tam VT, et al. Incidence and risk factors of retinopathy of prematurity from 2 neonatal intensive care units in a Hong Kong Chinese Population. Asia Pac J Ophthalmol (Phila) 2016;5(3):185–191. DOI: 10.1097/APO.0000000000000167.
12. Li ML, Hsu SM, Chang YS, et al. Retinopathy of prematurity in southern Taiwan: A 10-year tertiary medical center study. J Formos Med Assoc 2013;112(8):445–453. DOI: 10.1016/j.jfma.2012.03.002.
13. Lim ZD, Oo KT, Tai ELM, et al. Efficacy of WINROP as a screening tool for retinopathy of prematurity in the East Coast of Malaysia. Clin Ophthalmol 2020;14:1101–1106. DOI: 10.2147/OPTH.S247820.
14. Raffa LH, Alessa SK, Alamri AS, et al. Prediction of retinopathy of prematurity using the screening algorithm WINROP in a Saudi cohort of preterm infants. Saudi Med J 2020;41(6):622–627. DOI: 10.15537/smj.2020.6.25127.
15. Sute SS, Jain S, Chawla D, et al. Special focus on uvea and retina, original article use of an online screening algorithm – Weight, Insulin – derived growth factor 1, Neonatal Retinopathy of Prematurity (WINROP) for predicting retinopathy of prematurity in Indian preterm babies. Indian J Ophthalmol 2021;69(5):1214–1218. DOI: 10.4103/ijo.IJO_1521_20.
16. Noor MS, Elbarbary M, Embabi SN, et al. Screening and Risk Factors for Retinopathy of Prematurity in a Tertiary Care Hospital in Cairo. Clin Ophthalmol 2022;16:3257–3267. DOI: 10.2147/OPTH.S383493.
17. Lin Y, Chen S, Muo C. Risk of retinopathy of prematurity in preterm births with respiratory distress syndrome: A population-based cohort study in Taiwan. Int J Gen Med 2022;15:2149–2162. DOI: 10.2147/IJGM.S344056.
18. Ingolfsland EC, Haapala JL, Buckley LA, et al. Late growth and changes in body composition influence odds of developing retinopathy of prematurity among preterm infants. Nutrients 2019;12(1):78. Published 2019 Dec 27. DOI: 10.3390/nu12010078.
19. Klevebro S, Westin V, Sjöström E, et al. Early energy and protein intakes and associations with growth, BPD, and ROP in extremely preterm infants. Clin Nutr 2019;38(3):1289–1295. DOI: 10.1016/j.clnu.2018.05.012.
20. Pai HS, Joy R, Cherian V, et al. Anemia in relation to severity of retinopathy of prematurity in preterm babies born in tertiary care centre in South India. Int J Contemp Pediatr 2020;7(10):2005–2009. DOI: 10.18203/2349-3291.ijcp20204043.
21. Ge G, Zhang Y, Zhang M. Pregnancy-induced hypertension and retinopathy of prematurity: A meta-analysis. Acta Ophthalmol 2021;99(8):e1263–e1273. DOI: 10.1111/aos.14827.
22. Löfqvist C, Hansen-pupp I, Andersson E, et al. Validation of a new retinopathy of prematurity screening method monitoring longitudinal postnatal weight and insulin like growth factor I. Arch Ophthalmol 2009;127(5):622–627. DOI: 10.1001/archophthalmol.2009.69.
23. Ueda K, Miki A, Nakai S, et al. Prediction of severe retinopathy of prematurity using the weight gain, insulin-like growth factor 1, and neonatal retinopathy of prematurity algorithm in a Japanese population of preterm infants. Japanese J Ophthalmol 2020;64(2):223–227. DOI: 10.1007/s10384-019-00709-z.
24. Azad R, Chandra P, Patwardhan SD, et al. Profile of asymmetrical retinopathy of prematurity in twins. Indian J Ophthalmol 2010;58(3):209–211. DOI: 10.4103/0301-4738.62645.
25. Petriçli İS, Kara C, Işık DU, et al. Effect of birth weight on retinopathy of prematurity in discordant twin pairs. Indian J Ophthalmol 2019;67(6):806–810. DOI: 10.4103/ijo.IJO_1197_17.
26. Sanghi G, Dogra MR, Dutta S, et al. Intersibling variability of retinopathy of prematurity in twins and its risk factors. Int Ophthalmol 2012;32(2):113–117.

ORIGINAL RESEARCH

Comparison of Transcutaneous Bilirubin with Total Serum Bilirubin Levels Before, During, and Post-phototherapy in Preterm and Term Newborns in Uttarakhand, India

Ravi Sahota[1], Virender Singh[1], Heena Parveen[1], Navpreet Kaur[2], Neha Upreti[2], Abhay Mahindre[3], Bharat Bhushan Gupta[4]

Received on: 25 December 2023; *Accepted on*: 25 January 2024; *Published on*: 26 March 2024

Abstract

Background: Neonatal jaundice, although generally benign, can pose a significant threat to a select number of newborns, potentially resulting in severe brain damage or even death.

Objectives: To compare the transcutaneous bilirubin (TCB) with total serum bilirubin (TSB) levels before, during the phototherapy on a covered definite spot of skin and after 2 days of post-phototherapy in preterm and term newborns.

Materials and methods: A total of 272 babies, who had jaundice, admitted to NICU of Sahota Superspeciality Hospital, Kashipur, Uttarakhand, India, were enrolled in the study during the period March 2018 to February 2020. Transcutaneous bilirubin and TSB were done in all the babies and compared before starting phototherapy, during phototherapy, and after 48 hours of stopping the phototherapy.

Results: Before phototherapy (PT)-TCB showed statistically significant positive correlation with before PT-TSB. During PT-TCB showed statistically significant positive correlation with PT-TSB. After PT-TCB showed statistically significant positive correlation. Bilirubin level measured by TCB and TSB method was statistically significantly comparable before, during, and after PT ($p > 0.05$).

Conclusion: There is a significant positive correlation between TCB and TSB in preterm and term newborns who required phototherapy for hyperbilirubinemia, before starting the phototherapy, during phototherapy, and after 48 hours of its stoppage.

Clinical significance: Transcutaneous bilirubin is a good tool to do screening for hyperbilirubinemia. TCB can be used as a noninvasive tool to assess bilirubin during and after phototherapy.

Keywords: Bilirubin, Newborn, Phototherapy, Transcutaneous bilirubin, Total serum bilirubin.

Newborn (2024): 10.5005/jp-journals-11002-0086

Introduction

Hyperbilirubinemia is a common occurrence in both full-term and premature newborns, affecting approximately 60% of all newborns.[1] It is primarily a result of their increased bilirubin production and limited capacity to excrete it.[2] The majority of cases of newborn jaundice are mild and typically resolve on their own. However, in rare instances, infants may develop extremely high bilirubin levels, which can lead to a condition known as bilirubin encephalopathy and kernicterus which can result in high mortality and long-term complications, such as athetoid cerebral palsy, high-frequency hearing loss, and intellectual disability.[3,4] Early detection and appropriate management are crucial to prevent such complications. Estimating serum bilirubin levels through visual inspection of the skin is a quick and cost-effective method, but it is prone to errors, even when performed by experienced clinicians.[5]

Neonatal jaundice is a frequently-seen reason for hospital admission during the first week after birth and is primarily caused by hyperbilirubinemia.[6,7] Prompt monitoring of serum bilirubin levels and early intervention through methods like phototherapy or exchange blood transfusion can effectively prevent severe neonatal hyperbilirubinemia and its associated complications.[8]

Traditionally, total serum bilirubin (TSB) measured by a biochemical laboratory has been considered the gold standard for bilirubin level measurement. However, this method is invasive, involving needle pricks that carry infection risks and cause discomfort to neonates.[6] Additionally, the time required to obtain TSB results can delay the initiation of hyperbilirubinemia therapy.

[1]Department of Pediatrics and Neonatology, Sahota Superspeciality Hospital, Kashipur, Uttarakhand, India

[2]Department of Obstetrics and Gynecology, Sahota Superspeciality Hospital, Kashipur, Uttarakhand, India

[3]Department of Neonatology, Noble Hospital & Research Centre, Pune, Maharashtra, India

[4]Department of Pathology, Sahota Superspeciality Hospital, Kashipur, Uttarakhand, India

Corresponding Author: Ravi Sahota, Department of Pediatrics and Neonatology, Sahota Superspeciality Hospital, Kashipur, Uttarakhand, India, Phone: +91 7055020002, e-mail: sahota24@yahoo.com

How to cite this article: Sahota R, Singh V, Parveen H, *et al*. Comparison of Transcutaneous Bilirubin with Total Serum Bilirubin Levels Before, During, and Post-phototherapy in Preterm and Term Newborns in Uttarakhand, India. Newborn 2024;3(1):8–12.

Source of support: Nil

Conflict of interest: None

In recent years, transcutaneous bilirubinometers have emerged as a non-invasive alternative for estimating bilirubin levels. These portable devices use photometry to measure bilirubin levels by gently pressing a probe against the neonate's forehead or sternum. They provide immediate results, enabling swift treatment initiation and reducing the burden on healthcare providers.[9]

Current research regarding the accuracy of transcutaneous bilirubinometer measurements based on different skin locations

and time intervals after phototherapy is inconclusive.[10,11] Previous studies focused on various locations or time points separately and did not offer comprehensive results within a single study. Long waiting times may lead to inadequate phototherapy and treatment delays.[12] Therefore, the present study was conducted with aim to compare the transcutaneous bilirubin (TCB) with TSB levels before, during the phototherapy on a covered definite spot of skin and after 2 days of post-phototherapy in preterm and term newborns.

Materials and Methods

We performed a prospective observational study of all babies admitted to NICU of Sahota Superspeciality Hospital Kashipur, Uttarakhand, India during the period March 2018 to February 2020. During this study period, the 272 infants were divided in two groups: group A: Preterm babies ($n = 136$) and group B: term babies ($n = 136$).

The study included all preterm and term neonates with hyperbilirubinemia, who were admitted in the NICU satisfying the inclusion and exclusion criteria during the study period. The informed consent of the parents/relatives were taken before enrolling them in the study. The clinical profile of babies like demographic data, antenatal history, including relevant maternal diseases or complications during pregnancy, detailed history with feeding details, examination, and diagnosis was noted in a preformed pro forma. The discretion to use phototherapy for each case was taken by the attending neonatologist based on the hospital criteria. All babies were kept naked with diaper alone, to allow maximum skin exposure to phototherapy. Eye protection was used during the treatment. Phototherapy was given using ZEAL LED standard phototherapy units. Phototherapy was interrupted only for feedings breaks. It was stopped only once the TSB levels goes below the threshold of treatment. Total duration required for completion of phototherapy was documented in the information sheet of each patient.

Percentage was used to analyze data. The data were recorded in an Excel sheet and descriptive analysis was performed by Epi.Info. Sotware 7.2. Data were presented in the tables.

Material Used and Location Chosen

A circular photo-opaque patch around 2.5 cm in diameter, made from Maxicor electrode which was covered on one side with aluminum foil and the other side with an adhesive was used for covering the lower part of sternum.

Phototherapy Unit Used

Phototherapy unit was used in the treatment was zeal—LED continuous special neo blue light which emits a wavelength of 400–550 nm. Dominant wavelength range was 450–465 nm at peak irradiance of 45 μw/cm^2/nm.

TSB and TCB Estimation Techniques and Devices

All TSB values were obtained from intravenous samples of blood through fully automated spectrophotometer. TCB values were obtained using the latest version of Drager JM-105 which was the instrument used for it. This device was calibrated before usage according to manufacturer's recommendation.

Inclusion Criteria

All term and preterm newborn with hyperbilirubinemia. The exclusion criteria include parents or relatives who were not willing to give consent for research and neonates who received the exchange blood transfusion.

Table 1: Sociodemographic characteristic of study participants ($N = 272$)

Variable	*Number*	*%*
Gestational age (in week)		
<28	11	4.1
28.0–31.6	33	12.0
32.0–36.6	104	38.4
≥37.0	124	45.6
Mode of delivery		
Normal	129	47.4
LSCS	143	52.6
Gender		
Male	156	57.4
Female	116	42.6
Birth weight (in kg)		
<1.00	11	3.9
1.00–1.49	30	11.2
1.50–2.49	127	46.7
≥2.50	104	38.2
Time of passage of first stool (in hour)		
0–6	0	0.0
6–12	0	0.0
13–18	124	45.7
19–24	148	54.3
Duration of phototherapy (in hour)		
48	90	33.0
72	114	42.0
96	68	25.0

Results

Table 1 depicts that 4.1, 12.0, 38.4, and 45.6% of participants were noted with gestational age <28, 28–31, 32–36, and ≥37 weeks, respectively. Almost 47.4% of babies were delivered by normal vaginal delivery and 52.6% of babies were by LSCS. Around 57.4% of babies were male and 42.6% were female. Almost 3.9, 11.2, 46.7, and 38.2% participants noted with birth weight <1, 1–1.49, 1.50–2.49, ≥2.50 kg, respectively. Almost 0, 0, 45.7, and 54.3% of babies passed the first stool at 0–6, 6–12, 13–18, 19–24 hours after birth, respectively. Around 33, 42, and 25% of babies received phototherapy for 48, 72, and 96 hours, respectively.

Table 2 presents that the mean duration of phototherapy was 53.3 minutes with 12.9 SD and 79.2 minutes with 17.1 SD in babies who passed the first stool after birth at 13–18 and 19–24 hours, respectively. The difference in the meantime of phototherapy according to the time of passage of first stool was statistically significant ($p < 0.05$). The mean duration of phototherapy was 59.1 minutes with 18.1 SD and 70.3 minutes with 18.4 SD in babies whose weight loss after birth <5% and >5%, respectively. The difference in the meantime of phototherapy according to the weight loss was statistically significant ($p < 0.05$). The mean duration of phototherapy was 67.7 minutes with 18.9 SD and 69.9 minutes with 18 SD in babies delivered by normal and LSCS method, respectively. The difference in the meantime of phototherapy

Table 2: Association between 'Mean duration of PT' with different clinical parameters (*N* = 272)

Parameters	*PT (mean ± SD)*	*p-value*
Time of passage of first stool (in hour)		
13–18	53.3 ± 12.9	**0.001***
19–24	79.2 ± 17.1	
Weight loss (%)		
<5%	59.1 ± 18.1	**0.017***
>5%	70.3 ± 18.4	
Mode of delivery		
Normal	61.7 ± 18.9	0.248*
LSCS	69.9 ± 18.0	
Type of feeding		
Total parenteral nutrition (TPN)	74.4 ± 15.9	0.098**
Nasogastric tube (NGT)	63.3 ± 15.8	
Orogastric tube (OGT)	77.1 ± 21.0	
Breastfeeding (BF)	61.9 ± 14.9	

*Student's *t*-test, **One way ANNOVA test. *p*-values < 0.05 will be taken as significant

Table 3: Comparison of "mean bilirubin level" measured by TCB and TSB method at before, during, and after PT (*N* = 272)

	Bilirubin levels (mg/dL)		
PT	*TCB*	*TSB*	*p-value**
Before PT	14.62 ± 2.98	14.71 ± 3.0	**0.01**
During PT	7.49 ± 1.94	7.29 ± 1.91	**0.03**
After PT	11.52 ± 4.36	11.43 ± 4.39	**0.001**

*Student's *t*-test. *p*-values < 0.05 will be taken as significant

according to the mode of delivery was statistically not significantly different ($p > 0.05$). The mean duration of phototherapy was 74.4 minutes with 15.9 SD, 63.3 minutes with 15.8 SD, 77.1 minutes with 21.0 SD and 61.9 minutes with 14.9 SD in babies fed by TPN, NGT, OGT, and BF method, respectively. The difference in the meantime of phototherapy according to the type of feeding was statistically not significant ($p > 0.05$).

Table 3 depicts that the mean value of TCB was 14.62 mg/dL with 3.2 SD, 7.49 mg/dL with 1.94 SD, and 11.52 mg/dL with 4.36 SD and the mean value of TSB was 14.71 mg/dL with 3.0 SD, 7.29 mg/dL with 1.94 SD, and 11.43 mg/dL with 4.39 SD at before PT, during PT, and after PT, respectively. The difference in the mean value of TCB and TSB at before PT, during PT, and after PT was statistically significant ($p < 0.05$).

Table 4 illustrates that the mean value of TCB in preterm babies was 14.86 mg/dL with 3.2 SD, 6.85 mg/dL with 1.67 SD, and 10.13 mg/dL with 4.01 SD; and in term babies, it was14.29 mg/dL with 2.47 SD, 8.42 mg/dL with 1.94 SD, and 13.55 mg/dL with 4.08 SD at before PT, during PT, and after PT, respectively. The mean TSB values did not differ prior to initiation of phototherapy but were significantly different during and after phototherapy. The mean value of TSB in preterm babies was 14.96 mg/dL with 3.32 SD, 6.6 mg/dL with 1.59 SD, and 10.09 mg/dL with 3.31 SD; and in term babies, it was14.34 mg/dL with 2.47 SD, 8.28 mg/dL with 1.92 SD, and 13.45 mg/dL with 4.14 SD at before PT, during PT, and after PT, respectively. The difference in the mean value of TSB of preterm and term babies at during PT and after PT was statistically significant ($p < 0.05$) and statistically not significant at before PT ($p > 0.05$).

Table 4: Comparison of "mean bilirubin level according to gestational term" measured by TCB and TSB method at before, during, and after PT (*N* = 272)

	Bilirubin levels (mg/dL)		
PT	*Preterm*	*Term*	*p-value**
TCB			
Before PT	14.86 ± 3.2	14.29 ± 2.47	0.327
During PT	6.85 ± 1.67	8.42 ± 1.94	**0.001**
After PT	10.13 ± 4.01	13.55 ± 4.08	**0.001**
TSB			
Before PT	14.96 ± 3.32	14.34 ± 2.47	0.287
During PT	6.60 ± 1.59	8.28 ± 1.92	**0.001**
After PT	10.09 ± 3.32	13.45 ± 4.14	**0.001**

*Student's *t*-test. *p*-values < 0.05 will be taken as significant

Figure 1 shows that before PT-TCB showed statistically significant positive correlation with before PT-TSB with very high Pearson's *r*- value of 0.999 ($p < 0.05$). During PT-TCB showed statistically significant positive correlation with during PT-TSB with very high Pearson's *r*-value of 0.973 ($p < 0.05$). The after PT-TCB showed statistically significant positive correlation with after PT-TSB with very high Pearson's *r*-value of 0.999 ($p < 0.05$).

Discussion

In the present study, more than half of all cases (54%) were born prior to 37 weeks of gestational age. Fifty-two percent were delivered by lower segment cesarean section (LSCS). Because our center was a tertiary care referral center for mostly high-risk pregnancies, the incidence of LSCS births was significant in our study. Of the 272 cases studied, 57.4% were male and 42.6% were female. The birth weights of 46.7% of the 272 newborns tested ranged from 1.5 to 2.49 kg.

Fifty-four percent of infants in our study passed their first stool in the 18–24 hour period after birth. The overall duration of phototherapy was longer when stool transit was delayed. We discovered that the mean duration of phototherapy in these babies was 79 hours, compared to 59 hours in those who passed their first stool before 18 hours after birth. Meconium includes a high concentration of bilirubin, and its retention causes an increase in enterohepatic circulation, which contributes to neonatal hyperbilirubinemia.

The present study found that bilirubin level measured by TCB and TSB method was significantly comparable before, during, and after PT ($p > 0.05$). We found satisfactory results on the utility of TCB as a screening tool in our work, similar to those previously described. Our study further added that TCB is a reliable noninvasive tool in measurement of bilirubin during and after phototherapy when used to measure bilirubin in a covered site.

In this study, we discovered that the overall duration of phototherapy increased when the percentage of weight loss was more than 5%; the mean duration of phototherapy required in these babies was 70 hours as compared with those babies who had weight loss <5% requiring phototherapy for 59 hours of duration. These findings showed a statistically significant positive correlation. But we did not compare this with babies who had similar weight loss and had not developed hyperbilirubinemia. In the present study, there was no significant association between the mode of delivery and the type of feeding with the duration of phototherapy.

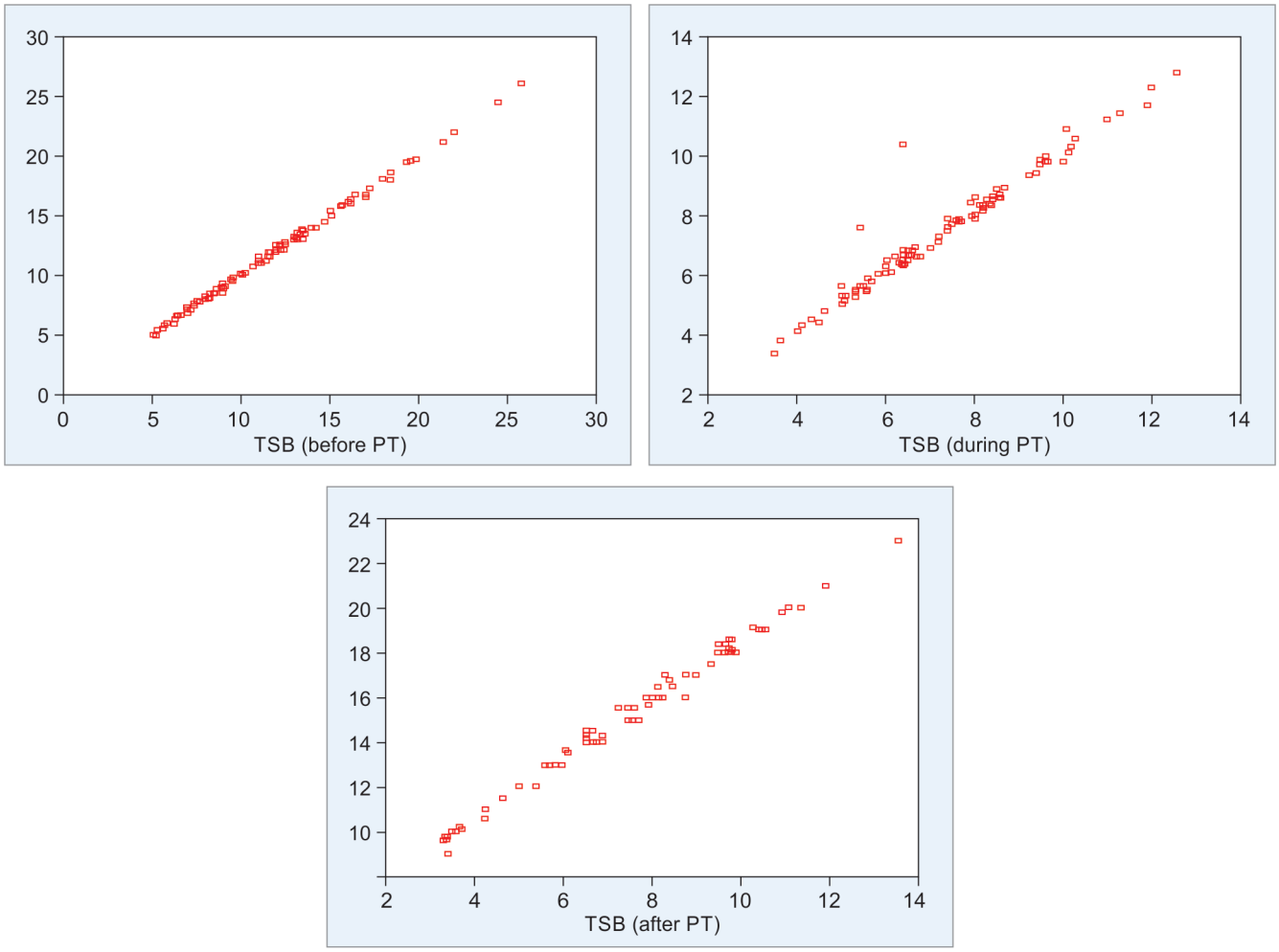


Fig. 1: Correlation between TCB and TSB method according to 'mean bilirubin level' at before, during and after PT ($N = 111$)

The present study revealed that the bilirubin level was significantly lower in preterm babies after starting of phototherapy and also after completion of phototherapy in both the TCB and TSB groups.

A 2017 prospective study conducted in the Haitian newborn population, in alignment with our findings, compared TCB and TSB levels during phototherapy. In this study, a good agreement was observed between the two methods. Transcutaneous bilirubin measurements could be a viable option for guiding jaundice treatment in regions where serum bilirubin tests are not widely available.[13]

Another study conducted in Iran partially supported our study's findings. It involved 134 full-term and 36 preterm newborns who received phototherapy and concluded that TCB measurements could be safely used to evaluate bilirubin levels in both preterm and full-term newborns receiving phototherapy, with slightly lower reliability in preterm newborns.[14] A meta-analysis in 2019 assessed the reliability of TCB measurements in premature newborns. It included 29 studies and concluded that TCB values obtained from the forehead and sternum regions were well correlated with TSB levels, making it a reliable method for assessing hyperbilirubinemia in premature newborns.[15] Furthermore, in a study by Vasava S and Dagli.[16] involving 306 full-term and preterm newborns, TCB measurements strongly predicted TSB levels across different gestational weeks and body regions. Similarly, Amneah A.[17] conducted a study with 80 newborns who received phototherapy by placing a patch on their skin. They reported a strong correlation between TCB values from the patched skin and TSB levels.

Conclusion

We noted a significant positive correlation between TCB and TSB in preterm and term newborns who required phototherapy for hyperbilirubinemia. Hence, in future, TSB measurements can be replaced by TCB measurements for monitoring bilirubin levels in neonates suffering from hyperbilirubinemia, as TCB measurement is a reliable, noninvasive method.

The overall duration of phototherapy increased when stool transit was delayed and percentage of weight loss was more than 5% but there is no significant correlation between other risk factors like mode of the delivery, type of feeding, and change in the color of the stool. All clinical parameters were assessed in babies already having hyperbilirubinemia. But these clinical parameters were not assessed and compared with healthy newborn.

Clinical Significance

Transcutaneous bilirubin is a good tool to do screening for hyperbilirubinemia. Transcutaneous bilirubin can be used as a noninvasive tool to assess bilirubin during and after phototherapy.

Ethical Approval

The study was approved by the Institutional Ethics Committee.

References

1. Woodgate P, Jardine LA. Neonatal jaundice: Phototherapy. BMJ Clin Evid 2015;2015:0319. PMID: 25998618.
2. Lauer BJ, Spector ND. Hyperbilirubinemia in the newborn. Pediatr Rev 2011;32(8):341–349. DOI: 10.1542/pir.32-8-341.
3. Sgro M, Campbell D, Shah V. Incidence and causes of severe neonatal hyperbilirubinemia in Canada. Can Med Assoc J 2006;175(6):587–590. DOI: 10.1503/cmaj.060328.
4. Ebbesen F, Andersson C, Verder H, et al. Extreme hyperbilirubinaemia in term and near-term infants in Denmark. Acta Paediatrica 2005;94(1):59–64. DOI: 10.1111/j.1651-2227.2005.tb01789.x.
5. Taylor JA. Discrepancies between transcutaneous and serum bilirubin measurements. Pediatrics 2015;135(2):224–231. DOI: 10.1542/peds.2014-1919.
6. Bhutani VK, Zipursky A, Blencowe H, et al. Neonatal hyperbilirubinemia and rhesus disease of the newborn: Incidence and impairment estimates for 2010 at regional and global levels. Pediatr Res 2013;74 Suppl 1(Suppl 1):86–100. DOI: 10.1038/pr.2013.208.
7. Grupp-Phelan J, Taylor JA, Liu LL, et al. Early newborn hospital discharge and readmission for mild and severe jaundice. Arch Pediatr Adolesc Med 1999;153(12):1283–1288. DOI: 10.1001/archpedi.153.12.1283.
8. Ministry of Health Malaysia. Clinical practice guidelines: Management of neonatal jaundice. 2nd ed. Kuala Lumpur, Malaysia: Ministry of Health; 2014.
9. Mohamed M, Ibrahim NR, Ramli N, et al. Comparison between the transcutaneous and total serum bilirubin measurement in Malay neonates with neonatal jaundice. Malays J Med Sci 2022;29(1):43–54. DOI: 10.21315/mjms2022.29.1.5.
10. Tan KL, Dong F. Transcutaneous bilirubinometry during and after phototherapy. Acta Paediatr 2003;92:327–331. PMID: 12725548.
11. Nagar G, Vandermeer B, Campbell S, et al. Effect of phototherapy on the reliability of transcutaneous bilirubin devices in term and near-term infants: A systematic review and meta-analysis. Neonatology 2016;109(3):203–212. DOI: 10.1159/000442195.
12. Casnocha LL, Matasova K, Zibolen M, et al. Accuracy of transcutaneous bilirubin measurement in newborns after phototherapy. J Perinatol 2016;36(10):858–861. DOI: 10.1038/jp.2016.91.
13. Johnson SM, Vasu V, Marseille C, et al. Validation of transcutaneous bilirubinometry during phototherapy for detection and monitoring of neonatal jaundice in a low-income setting. Paediatr Int Child Health 2020;40(1):25–29. DOI: 10.1080/20469047.2019.1598126.
14. Radfar M, Hashemieh M, Shirvani F, et al. Transcutaneous bilirubinometry in preterm and term newborn infants before and during phototherapy. Arch Iran Med 2016;19(5):323–328. PMID: 27179163.
15. Hassan Shabuj M, Hossain J, Dey S. Accuracy of transcutaneous bilirubinometry in the preterm infants: A comprehensive metaanalysis. J Matern Fetal Neonatal Med 2019;32(5):734–741. DOI: 10.1080/14767058.2017.1390561.
16. Vasava S, Dagli P. Transcutaneous bilirubinometry: Influence of gestational age and measurement site. Sri Lanka J Child Health 2018;47:27–32. DOI: 10.4038/SLJCH.V47I1.8426.
17. Amneenah A. Comparison of diagnostic accuracy of transcutaneous bilirubinometry during phototherapy by using skin patch. Int J Health Sci 2022;6:7276–7288. DOI: 10.53730/ijhs.v6nS2.6779.

ORIGINAL RESEARCH

Timely Respiratory Support Can Improve Clinical Outcomes of Premature Infants in a Country with Limited Medical Resources due to Chronic Conflicts

Numan N Hameed[1], Musaab M Khaleel[2], Ola D Saugstad[3]

Received on: 22 December 2023; *Accepted on*: 31 January 2024; *Published on*: 26 March 2024

Abstract

Introduction: The children of Iraq have suffered greatly from military conflicts and economic sanctions since 1991. Recent years have shown some improvement in neonatal and infant mortality but more efforts are needed; prematurity and associated respiratory distress syndrome (RDS) remain the two leading causes. In this study, we investigated the efficiency of timely institution of nasal continuous positive airway pressure (nCPAP) in stabilizing these infants. These data are needed for the optimum allocation of financial resources to improve the healthcare outcomes of infants.

Patients and methods: This prospective cross-sectional study was carried out over 6 months from April 1st to September 30th, 2022. Inborn preterm infants born between 26 and 32^{+0} weeks' gestation who required respiratory support after delivery or immediately after admission were included. The data for the initial course of respiratory support and outcomes were assessed.

Results: In our cohort of 123 infants, nCPAP significantly increased the likelihood of clinical stabilization in infants with a gestational age (GA) >28 weeks ($p = 0.022$), birth weight (BW) ≥1500 gm ($p = 0.016$), use of antenatal steroids ($p = 0.002$), Apgar score at 5 minutes of life ($p = 0.022$), mild radiographic findings ($p = 0.007$), and sepsis without prolonged rupture of membranes ($p = 0.027$). Nasal continuous positive airway pressure also reduced the need for surfactant ($p = 0.001$) and mortality ($p = 0.0001$).

Conclusion: Early institution of nCPAP improved the respiratory status of premature infants who were born at a gestational age from >28 to ≤32 weeks, had birth weight ≥1500 gm, had received antenatal steroids, had a 5-minute Apgar score >7, and had sepsis but no PROM. The success of early nCPAP reduced the need for surfactant and mechanical ventilation, and the risk of pulmonary hemorrhage and mortality.

Keywords: Baby, Infant, INSURE, Mechanical ventilation, Minimal invasive surfactant therapy, Neonatology, Neonate, Neonatal intensive care unit, Newborn, Preterm infants, Respiratory distress syndrome.

Newborn (2024): 10.5005/jp-journals-11002-0088

Introduction

Iraq is a middle-income country with a total population of 44.5 million. A large proportion of this community is relatively young; 20 million (45%) are under the age of 15 years (y) and 3.9 million (17%) are <5 years. There are 1.2 million annual births, and as in any other country, children are a relatively high-risk section of its population. The children here suffered considerably during the Gulf War of 1991 and its aftermath.[1–4] An estimated 46,000 Iraqi children were killed during the wars beginning in 1991, and many more were lost in subsequent conflicts and economic sanctions; the negative healthcare sequelae of these conflicts can still be seen after two decades.[1,5,6]

In a panoramic view, Iraq is a country with moderately-high neonatal and childhood mortality. Notably, 58% of all <5 year deaths occur in neonates.[7] In the 1980s, the average perinatal mortality rate (PMR) in Iraq was about 28 per 1,000 live births. However, following the onset of military conflicts during the 1990–1999 decade, the average PMR rose to 107 per 1,000 live births. The most important causes of neonatal deaths include prematurity (9.1% of all births) and its complications related to pulmonary immaturity and infections (47% of all neonatal deaths), birth asphyxia (20%), and congenital anomalies (17%).

Recent years have shown improved social safety and there has been some slow improvement in healthcare outcomes. During the period 1990–2020, the neonatal mortality has decreased from 26 to about 15/1,000 live births, and the infant mortality rate from 42 to 21.[8] The most frequently noted causes of neonatal deaths are still low birth weight, prematurity, perinatal infections, and birth asphyxia.[9] Children, per international conventions, ought to be

[1]Department of Pediatrics, College of Medicine, Baghdad University; Department of Pediatrics, Children Welfare Teaching Hospital, Medical City Complex, Baghdad, Iraq

[2]Department of Pediatrics, Children Welfare Teaching Hospital, Medical City, Baghdad; Department of Pediatrics, College of Medicine, Ninevah University, Iraq

[3]Department of Pediatric Research, University of Oslo, Oslo; Institute of Oral Biology, University of Oslo, Norway

Corresponding Author: Numan N Hameed, Department of Pediatrics, College of Medicine, Baghdad University; Department of Pediatrics, Children Welfare Teaching Hospital, Medical City Complex, Bab Al-Muadham, Baghdad, Iraq, Phone: +096 47901753286, e-mail: numanalhamdani@yahoo.com

How to cite this article: Hameed NN, Khaleel MM, Saugstad OD. Timely Respiratory Support Can Improve Clinical Outcomes of Premature Infants in a Country with Limited Medical Resources due to Chronic Conflicts. Newborn 2024;3(1):13–18.

Source of support: Nil

Conflict of interest: None

protected from social adversities during armed conflicts but the efforts have not quite sufficed. Recent data show that despite all improvements, only 26.9% of all deliveries are currently attended by skilled birth attendants.[10]

In premature infants, respiratory distress syndrome (RDS) is the leading cause of morbidity and mortality in this region. Due to limitations in resources, we have stressed preventive and pre-emptive treatment such as with timely application of nasal-continuous positive airway pressure (nCPAP). If the work of breathing is high and there is an increasing need for oxygen, surfactant is administered as soon as possible.[11] Prenatally, a single course of corticosteroids is offered to women at risk of preterm delivery if they complete <34 weeks' gestation when pregnancy begins to be considered potentially viable. This treatment is administered ideally at least 24 hours (h) prior to delivery. This dose of corticosteroids improves survival and reduces the risk of RDS, necrotizing enterocolitis (NEC), and intraventricular hemorrhage (IVH).[11,12]

According to the European Consensus Guidelines, all spontaneously-breathing preterm infants with RDS should have access to CPAP for clinical management.[11] Intubation should be reserved for babies not responding to positive pressure ventilation via face masks or nasal prongs.[13] Surfactant therapy improves survival and reduces pneumothoraces, and therefore, is an important part of the management of RDS. Early initiation of CPAP may help prevent the harmful effects of intubation and mechanical ventilation (MV) during the transitional phase. The INtube, SURfactant, Extubate (IN-SUR-E) technique, involving surfactant bolus administration followed by brief bag ventilation and rapid extubation without ongoing ventilation, may also reduce lung injury.[14] Currently, the best-accepted method is using the less invasive surfactant administration (LISA) or minimally invasive surfactant technique (MIST).[15,16] If these modalities do not succeed, MV is used cautiously as a rescue modality to achieve "acceptable" alveolar function and prevent acidosis.[15] A previous Iraqi study at our center in 2013 showed a high failure rate of CPAP. In a cohort of 70 neonates, 37 (52.9%) failed CPAP, and 29 (78.3%) had to be rescued using MV.[17] In the present study, we re-assessed the impact of nCPAP as the initial modality for respiratory support and assessed the clinical outcomes in preterm infants born at a gestational age (GA) between 26 and 32^{+0} weeks. These data are important to optimize the allocation of financial resources to improve infant healthcare outcomes in an underserved area with longstanding conflicts and limited resources.

Patients and Methods

This single-center, prospective, cross-sectional study included 123 preterm (26–32 weeks' gestation) over 6 months (April 1, 2022–September 30, 2022) in the level 3 neonatal intensive care unit (NICU) of Baghdad Teaching Hospital/Medical City complex. This NICU is equipped with 20 incubators (Dräger and Atom), 22 monitors, 15 CPAP (SLE1000), and (medin CNO), which involve all modes of noninvasive ventilation (CPAP, nasal high-frequency oscillatory ventilation, nasal intermittent positive pressure ventilation, and synchronized nasal intermittent positive pressure ventilation). In addition, there are 9 ventilators SLE (4000, 5000) with the 3 main modes of ventilation (synchronized intermittent mandatory ventilation, assist/control ventilation, and pressure support ventilation). The unit has ready access to a blood gas analysis machine.

The inclusion criteria were: (A) GA of 26–32^{+0} weeks; (B) RDS, with at least two of the following findings: Tachypnea (respiratory rate >60/min), dyspnea (increased respiratory effort with nasal flaring expiratory grunting, and intercostal retractions); (C) need of supplementary oxygen; and (D) need of surfactant administration.[18,19] Exclusion criteria were birth defects affecting the respiratory system or other major congenital anomalies.

Data collected retrospectively from the records were: age, sex, GA, birth weight (BW), mode of delivery, being small for GA (BW <10th centile), maternal history of hypertension, diabetes mellitus, preeclampsia, prolonged rupture of membranes (PROM) >18 hours, antenatal steroids use, Apgar score at 1 and 5 minutes, intubation in the delivery room, use of CPAP as initial respiratory support, need for MV as initial respiratory support, use of surfactant, radiographic features of RDS, length of hospital stay (LOS), and caffeine supplementation.

Short-term outcomes included a change from CPAP to MV, pneumothorax, pulmonary hemorrhage, bronchopulmonary dysplasia (BPD), sepsis, successful management with initial CPAP without a need to change to MV, and death. GA was calculated based on the mothers last menstrual period and/or either an early pregnancy ultrasound scan or a New Ballard score. The babies were initially treated with CPAP (5–7 cm H_2O) and FiO_2 of 0.3. Exogenous surfactant was administered through an endotracheal tube applying the INSURE technique. Indications for surfactant include preterm with RDS who fail nCPAP and require intubation and MV. Surfactant therapy was administered to infants who required FiO_2 >0.3–0.4 to maintain SpO_2 >90 percent despite the use of nCPAP or X-ray showing severe RDS (white lung) and if a preterm baby <30 weeks required intubation for stabilization. LISA use was not documented in any of the neonatal care units in Iraq. Based on radiological findings, the severity of RDS was graded as mild (mild granularity of lungs), moderate (generalized granularity of lungs with air bronchogram with preserved cardiac borders), and severe (opaque 'white-out' lungs with loss of cardiac borders).[20]

Continuous positive airway pressure failure was defined as the need for MV during the 1st week for ≥1 of the following indications: (A) no spontaneous breathing or frequent apnea; (B) marked retractions and/or respiratory rates ≥90 per minute; and (C) FiO_2 >0.4 to maintain a target SpO_2 of 90–94%.[21]

Statistics

Statistical analysis of data was carried out using the software package [Statistical Packages for Social Sciences (SPSS)], version 28. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of the difference of different percentages (qualitative data) was tested using the Pearson Chi-square test with the application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the *p*-value was ≤ 0.05.[22–25]

Ethics

Patients were included after verbal parental consent. The identity of patients was kept anonymous. The ethical committees at the Children's Welfare Teaching Hospital and Baghdad Teaching hospital, Medical City approved the studies.

Results

123 preterm babies were included. The mean (± standard deviation, SD) of GA was 29.8 ± 1.6 weeks. Eleven (8.9%) had a GA <28 weeks and 112 (91.1%) were born between 28 and 32 weeks. Eighty (65.0%) were males. The mean BW was 1247.5 ± 291.8 gm; 38 (30.9%)

Table 1: Association between demographic characteristics and outcome of early CPAP (n = 103)

	CPAP prognosis (n = 103)				
	Failure n = 38		*Success n = 65*		
	No	*%*	*No*	*%*	*p-value*
Gestational age (weeks)					
<28 weeks	7	18.4	3	4.6	0.022*
28–32 weeks	31	81.6	62	95.4	
Mean ± SD	29.5 ± 1.9		30.3 ± 1.4		
Sex					
Female	11	28.9	21	32.3	0.722
Male	27	71.1	44	67.7	
Birth weight (gms)					
<1000 gm	8	21.1	3	4.6	0.016*
1000–1500 gm	21	55.3	35	53.8	
>1500 gm	9	23.7	27	41.5	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at <0.05 level

Table 2: Association between maternal risk factor and outcome of early CPAP use

	CPAP prognosis				
	Failure n = 38		*Success n = 65*		
	No	*%*	*No*	*%*	*p-value*
Mode of delivery					
CS	29	73.7	57	89.2	0.113
NVD	9	26.3	8	10.8	
Small for gestational age					
Yes	5	13.2	6	9.2	0.534
No	33	86.8	59	90.8	
Maternal history hypertension					
Yes	11	28.9	25	38.5	0.329
No	27	71.1	40	61.5	
DM					
Yes	6	15.8	7	10.8	0.459
No	32	84.2	58	89.2	
Preeclampsia					
Yes	3	7.9	2	3.1	0.272
No	35	92.1	63	96.9	
Use of antenatal steroid					
Yes	23	60.5	61	93.8	0.0001*
No	15	39.5	4	6.2	
Prolong rupture of membrane >18 hours					
Yes	15	39.5	12	18.5	0.019 *
No	23	60.5	53	81.5	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at <0.05 level

Table 3: Association between Apgar score at 1 and 5 minutes and outcome of early CPAP

	CPAP prognosis				
	Failure n = 38		*Success n = 65*		
	No	*%*	*No*	*%*	*p-value*
Apgar score at 1 minute					
Poor (<7)	37	97.4	60	92.3	0.290
Good (≥7)	1	2.6	5	7.7	
Apgar score at 5 minutes					
Poor (<7)	**7**	**18.4**	**3**	**4.6**	**0.022***
Good (≥7)	31	81.6	62	95.4	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at <0.05 level

weighed ≥1500 gm, 67 (54.5%) between 1000 and 1500 gm, and 18 (14.6%) weighed <1000 gm. One hundred and two (82.9%) neonates were delivered by emergency cesarean sections, whereas 21 (17.1%) were delivered by normal vaginal deliveries. Fourteen (11.4%) cases were SGA. Ninety-four (76.4%) mothers received 1 course of antenatal steroids. The frequency of the need for respiratory support vis-à-vis the incidence of maternal risk factors was maternal hypertension (42, 34.1%), diabetes (14, 11.4%), preeclampsia (6, 4.9%), and prolonged (>18 hours) rupture of membranes (36, 29.3%).

At 1 minute, 117 infants (95.1%) had an Apgar score of <7 and 6 (4.9%) had ≥7. At 5 minutes, 23 (18.7%) had Apgar <7, and 100 (81.3%) had ≥ 7. Thirty (24.4%) were intubated in the delivery room, 103 (83.7%) were treated with early CPAP, and 20 (16.3%) needed early rescue MV. Thirty-five (28.5%) received surfactant and 101 (82.1%) were started on caffeine. Eighty-eight (71.5%) had radiographic changes of mild RDS, 12 (9.8%) had moderate, and 23 (18.7%) had severe changes. The mean LOS was 10.3 ± 6.8 (range 1–32) days. Twenty-seven (22%) stayed for ≤7 days, 67 (54.5%) for 8–14, 16 (13%) for 15–20, and 13 (10.6%) for >20 days.

Thirty-eight (30.8%) infants needed rescue MV after failure of CPAP. Twenty-six (21.1%) developed pulmonary hemorrhage, 6 (4.9%) had pneumothorax, 35 (28.5%) had sepsis, and 2 (1.6%), developed BPD. Twenty-three (18.7%) were referred to a tertiary pediatric referral hospital to continue treatment or failure to thrive.

Overall, 80 (65%) infants survived, and 43 (35%) died. One hundred and three (83.7%) infants received early CPAP but 38 (30.8%) had to be changed to MV. There were significant associations between GA (p = 0.022), BW (p = 0.016), and outcome of early CPAP, however, there was no significant association between gender (p = 0.722) and outcome of early CPAP. The outcome of early CPAP showed significant associations with the use of antenatal steroids (p = 0.002) and Apgar score at 5 minutes (p = 0.022). There was a higher risk of failure of CPAP if there was sepsis with prolonged (>18 hours) rupture of membranes (p = 0.019). There were no associations with maternal history (hypertension, diabetes, and preeclampsia), the mode of delivery (p = 0.113), SGA (p = 0.53), and Apgar score at 1 minute (p = 0.29; Tables 1 to 3). There was a significant association between the success of early CPAP with the use of surfactant (p = 0.001), mild X-ray findings (p = 0.007), pulmonary hemorrhage, sepsis (p = 0.027), and being discharged well (p = 0.0001). There was no significant association between pneumothorax, BPD, referral, and success of early CPAP (Table 4).

Discussion

Our understanding of the optimum, early application of CPAP in the respiratory management of preterm infants is still developing.[26] In our cohort, 103 (83.7%) were treated with early CPAP. The enthusiasm for initial CPAP therapy is often tempered by concerns about the

Table 4: Association between diagnosis, treatment and outcome of early CPAP use

	CPAP prognosis				
	Failure n = 38		*Success n = 65*		
	No	*%*	*No*	*%*	*p-value*
Use of surfactant					
Yes	**13**	**34.2**	**5**	**7.7**	**0.001***
No	25	65.8	60	92.3	
X-ray finding					
Mild	**26**	**68.4**	**60**	**92.3**	**0.007***
Moderate	2	5.3	1	1.5	
Severe	10	26.3	4	6.2	
Pneumothorax					
Yes	3	7.9	2	3.1	0.272
No	35	92.1	63	96.9	
Pulmonary hemorrhage					
Yes	**16**	**42.1**	–	–	**0.0001***
No	22	57.9	65	100.0	
Sepsis					
Yes	**16**	**42.1**	**14**	**21.5**	**0.027***
No	22	57.9	51	78.5	
Bronchopulmonary dysplasia					
Yes	1	2.6	1	1.5	0.698
No	37	97.4	64	98.5	
Referral					
Yes	4	10.5	17	26.2	0.057
No	34	89.5	48	73.8	
Outcome					
Improved and discharged well	**11**	**28.9**	**65**	**100.0**	**0.0001***
Dead	27	71.1	–	–	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at <0.05 level

possible consequences of delays in surfactant administration and loss of efficacy of this relatively expensive treatment modality. These findings resemble those reported by Rehman et al. ($p < 0.00001$),[27] but not those of Celik et al.[19] who did not detect this association ($p = 0.118$). These differences could be related to differences in GA in the two cohorts. Celik and coworkers had studied a less mature cohort with a GA <32 weeks.[19] The average BW in our cohort resembled that of Rehman et al.[27] but not with the patients enrolled by Pillai and colleagues, who did not find an association.[28]

In our study, there was a significant association between the use of antenatal steroids and the success of early CPAP. These data agree with those reported by Tavares et al.[29] and Arora et al.[30] Similarly, there was a strong association between PROM and the need for early CPAP, which was consistent with the observations of Pillai and coworkers.[28] We did not find any relationship between Apgar scores at 1 minute and the use of early CPAP. Contrary to the findings noted by Celik et al.[19] our outcomes were better when the 5-minute Apgar scores were higher. There might have been some differences in delivery room resuscitation care.

Similar to the reports of Vieira et al.[31] and Tavares et al.[29] infants treated with early CPAP were less likely to need surfactant therapy, Similarly, more infants treated with early CPAP showed milder radiographic findings of RDS. These findings could indicate that early initiation of CPAP prevented the progression of RDS, but it is also possible that those who had milder respiratory disease were more likely to remain stable with timely institution of CPAP and did not need surfactant and/or MV. These findings resemble those reported by the groups led by both Rehman et al. and Pillai et al.[27,28]

The high incidence of pulmonary hemorrhage in our group likely indicated a higher severity of RDS in our cohort. Other possibilities, such as the protocol for surfactant administration, and the MV settings, could also have played a role and need to be reviewed. Similar to the findings of Boix et al.[32] fewer infants who received early CPAP had a pulmonary hemorrhage. Sepsis and pneumonia also affected the efficacy of early CPAP. These findings are consistent with those reported by the groups led by Koti et al.[33] and Pillai et al.[28] We had a high mortality rate in infants with sepsis. The incidence of BPD was low, but we did not have any infants born prior to 26 weeks gestation who would have been at higher risk of developing chronic lung changes.

In our cohort, preterm infants who were born ≤32 weeks and were treated with early CPAP, (73.8%) improved and were discharged with good outcomes. There was a significant association between improvement and decreased mortality with early CPAP use and these data are in agreement with the teams led by Arora et al.[30] and Hameed et al.[17] who work on the same campus. However, Celik et al.[19] did not find a similar reduction in mortality.

We need more studies in larger cohorts to determine whether our findings will stay consistent in those settings. There is a need for authentic systems for risk assessment with home deliveries, documentation of delays in patient referral, frequencies of hospital readmissions, and access to outpatient care after discharge or in other hospitals. In our conflict-affected regions, the availability of supportive treatment modalities such as blood transfusions in infants with severe anemia of prematurity may also be important.

In a previous report, we have described how access to medical facilities can affect infant outcomes in war zones. Adverse security situations and difficulties in transportation can be important limiting factors. Difficulties in reaching the hospital in a timely fashion, blockage of roads, disruption of infrastructure, and personal constraints can prevent families from seeking medical attention in a timely fashion.[34] The constraints that prevent families from transporting a sick infant to a medical facility also affect the healthcare workers. It is difficult to imagine even after reaching a medical facility, how disheartening it can be to find out that the needed healthcare workers could not make it to the hospital on that day or might never make it. Medical supplies might also be inadequate in quantity or quality, and limitations in the skills of available healthcare providers can be important limiting factors. Some infants may have to be delivered at home by untrained personnel or receive only minimal birthing care in hospitals.

Morbidity and mortality are not only related to the direct effect of an armed conflict but also diminishing medical resources. Reduction of neonatal and child mortality is lagging behind the resolution of armed conflicts. In countries like Iraq, and countries with similar situations such as Yemen, Afghanistan, Mali, and Somalia, neonatal mortalities are among the highest in the world. The differences are appalling when compared to neonatal mortality rates of ≤5 per 1,000 live births in the more peaceful Western countries.[35] In our study, the high mortality and morbidities including the poor outcomes of respiratory support in preterm

infants <32 weeks may be related to the maternal-child healthcare system being hampered by many years of long-term conflicts.[36] These data emphasize the need for a global, synergistic quest for solutions.[37]

Conclusion

The study shows that GA >28 and ≤32 weeks, BW ≥1500 gm, use of antenatal steroids, 5-minute Apgar scores >7, and sepsis with absence of PROM >18 hours prior to delivery were associated with significant benefits of early CPAP. The respiratory management of preterm infants with success of early CPAP reduced the need for surfactant and reduced the incidence of complications such as pulmonary hemorrhage. Early CPAP also reduced mortality.

Recommendations

In our conditions, antenatal corticosteroids are advisable for all women at risk of preterm delivery prior to 34 weeks gestation. There is a need to prevent and treat sepsis in a timely fashion and initiate CPAP as early as possible in premature infants with RDS to prevent pulmonary hemorrhage, need for surfactant, and reduce mortality. Further studies are recommended to evaluate the effect of new techniques of surfactant administration such as LISA in infants in high-risk war zones such as Iraq.

Orcid

Numan N Hameed https://orcid.org/0000-0001-5987-1693

References

1. Ascherio A, Chase R, Cote T, et al. Effect of the Gulf War on infant and child mortality in Iraq. N Engl J Med 1992;327(13):931–936. DOI: 10.1056/NEJM199209243271306.
2. Akunjee M, Ali A. Healthcare under sanctions in Iraq: An elective experience. Med Confl Surviv 2002;18(3):249–257. DOI: 10.1080/13623690208409633.
3. Nerlander MP, Leidman E, Hassan A, et al. Fatalities from firearm-related injuries in selected Governorates of Iraq, 2010–2013. Prehosp Disaster Med 2017;32(5):548–555. DOI: 10.1017/S1049023X17006495.
4. Awqati NA, Ali MM, Al-Ward NJ, et al. Causes and differentials of childhood mortality in Iraq. BMC Pediatr 2009;9:40. DOI: 10.1186/1471-2431-9-40.
5. Ali MM, Shah IH. Sanctions and childhood mortality in Iraq. Lancet 2000;355(9218):1851–1857. DOI: 10.1016/S0140-6736(00)02289-3.
6. Al-Nouri L, Al-Rahim Q. The effect of sanctions on children of Iraq. Arch Dis Child 2003;88(1):92. DOI: 10.1136/adc.88.1.92.
7. UNICEF. Monitoring the situation of children and women: Iraq. 2024. Available from: https://data.unicef.org/countdown-2030/country/Iraq/1/.
8. The World Bank, UNICEF. Mortality rate, neonatal (per 1,000 live births). Available from: https://data.worldbank.org/indicator/SH.DYN.NMRT.
9. Nasheit NA. Perinatal and neonatal mortality and morbidity in Iraq. J Matern Fetal Neonatal Med 2003;13(1):64–67. DOI: 10.1080/jmf.13.1.64.67.
10. Siziya S, Muula AS, Rudatsikira E. Socio-economic factors associated with delivery assisted by traditional birth attendants in Iraq, 2000. BMC Int Health Hum Rights 2009;9:7. DOI: 10.1186/1472-698X-9-7.
11. Sweet DG, Carnielli VP, Greisen G, et al. European consensus guidelines on the management of respiratory distress syndrome: 2022 Update. Neonatology 2023;120(1):3–23. DOI: 10.1159/000528914.
12. Roberts D, Brown J, Medley N, et al. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev 2017;3(3):CD004454. DOI: 10.1002/14651858.CD004454.pub3.
13. Norman M, Jonsson B, Wallstrom L, et al. Respiratory support of infants born at 22–24 weeks of gestational age. Semin Fetal Neonatal Med 2022;27(2):101328. DOI: 10.1016/j.siny.2022.101328.
14. Stevens TP, Harrington EW, Blennow M, et al. Early surfactant administration with brief ventilation vs. selective surfactant and continued mechanical ventilation for preterm infants with or at risk for respiratory distress syndrome. Cochrane Database Syst Rev 2007;2007(4):CD003063. DOI: 10.1002/14651858.CD003063.pub3.
15. Klingenberg C, Wheeler KI, McCallion N, et al. Volume-targeted versus pressure-limited ventilation in neonates. Cochrane Database Syst Rev 2017;10(10):CD003666. DOI: 10.1002/14651858.CD003666.pub4.
16. Abdel-Latif ME, Davis PG, Wheeler KI, et al. Surfactant therapy via thin catheter in preterm infants with or at risk of respiratory distress syndrome. Cochrane Database Syst Rev 2021;5(5):CD011672. DOI: 10.1002/14651858.CD011672.pub2.
17. Hameed NN, Abdul Jaleel RK, Saugstad OD. The use of continuous positive airway pressure in preterm babies with respiratory distress syndrome: A report from Baghdad, Iraq. J Matern Fetal Neonatal Med 2014;27(6):629–632. DOI: 10.3109/14767058.2013.825595.
18. Elgin TG, Berger JN, Thomas BA, et al. Ventilator management in extremely preterm infants. Neoreviews 2022;23(10):e661–e676. DOI: 10.1542/neo.23-10-e661.
19. Celik M, Bulbul A, Uslu S, et al. A comparison of the effects of invasive mechanic ventilation/surfactant therapy and non-invasive nasal-continuous positive airway pressure in preterm newborns. J Matern Fetal Neonatal Med 2018;31(24):3225–3231. DOI: 10.1080/14767058.2017.1367380.
20. Ainsworth SB. Pathophysiology of neonatal respiratory distress syndrome: Implications for early treatment strategies. Treat Respir Med 2005;4(6):423–437. DOI: 10.2165/00151829-200504060-00006.
21. Keszler M, Suresh G. Goldsmith's Assisted Ventilation of the Neonate. 7th edition. Keszler M, Suresh G, Goldsmith JP (Eds). Elsevier Health Sciences, Philadelphia; 2022. pp. 224, 235–237.
22. Celentano DD, Szklo M. Gordis Epidemiology. 6th edition. Elsevier, Barcellona; 2020, pp. 179–215.
23. Daniel WW, Cross CL. Biostatistics: A Foundation for Analysis in the Health Sciences 10th edition: John Wiley & Sons, Inc.; USA; 2013. pp. 600–670.
24. Moore DS, McCabe G. Introduction to the Practice of Statistics. 9th ed New York: WH Freeman and Company; 2009. pp. 900–1004.
25. Dawson B, Trapp RG. Basic & Clinical Biostatistics: Lange Medical Books; McGrawHill, USA; 2004. pp. 24–162.
26. Dargaville PA, Gerber A, Johansson S, et al. Incidence and outcome of CPAP failure in preterm infants. Pediatrics 2016;138(1):e20153985. DOI: 10.1542/peds.2015-3985.
27. Rehman A, Quddusi AI, Nadeem A, et al. Early nasal continuous positive airway pressure in preterm neonates with Respiratory Distress Syndrome. Professional Med J 2021;28(7):957–962. DOI: 10.29309/TPMJ/2021.28.07.5707.
28. Pillai MS, Sankar MJ, Mani K, et al. Clinical prediction score for nasal CPAP failure in pre-term VLBW neonates with early onset respiratory distress. J Trop Pediatr 2011;57(4):274–279. DOI: 10.1093/tropej/fmq047.
29. Tavares PN, Flor-de-Lima F, Soares H, et al. Early nCPAP versus intubation in very low birth weight infants. J Pediatr Neonat Individual Med 2013;2(2):1–6. DOI: 10.7363/64.
30. Arora V, Gediya SG, Jain R. Outcome of premature babies with RDS using bubble CPAP. Int J Contemp Pediatr 2017;4(3):939–942. DOI: 10.18203/2349-3291.ijcp20171702.
31. Vieira B, Souza TR, Paschoal LN, et al. Early CPAP protocol in preterm infants with gestational age between 28 and 32 weeks: Experience of a public hospital. Braz J Phys Ther 2021;25(4):421–427. DOI: 10.1016/j.bjpt.2020.09.001.
32. Boix H, Fernandez C, Serrano Martin MDM, et al. Failure of early non-invasive ventilation in preterm infants with respiratory distress

syndrome in current care practice in Spanish level-III neonatal intensive care units-A prospective observational study. Front Pediatr 2023;11:1098971. DOI: 10.3389/fped.2023.1098971.
33. Koti J, Murki S, Gaddam P, et al. Bubble CPAP for respiratory distress syndrome in preterm infants. Indian Pediatr 2010;47(2):139–143. DOI: 10.1007/s13312-010-0021-6.
34. Hameed NN, Na' Ma AM, Vilms R, et al. Severe neonatal hyperbilirubinemia and adverse short-term consequences in Baghdad, Iraq. Neonatology 2011;100(1):57–63. DOI: 10.1159/000321990.
35. Saugstad OD, Modi N, Moretti C, et al. Newborns and children in war and terror. Neonatology 2023:1–4. DOI: 10.1159/000535401.
36. Hameed NN, Hussein MA. BIND score: A system to triage infants readmitted for extreme hyperbilirubinemia. Semin Perinatol 2021;45(1):151354. DOI: 10.1016/j.semperi.2020.151354.
37. Lawn JE, Bhutta ZA, Ezeaka C, et al. Ending preventable neonatal deaths: Multicountry evidence to inform accelerated progress to the sustainable development goal by 2030. Neonatology 2023;120(4):491–499. DOI: 10.1159/000530496.

REVIEW ARTICLE

Milk Fat Globules: 2024 Updates

Akhil Maheshwari[1,2], Harshvardhan Mantry[3], Nitasha Bagga[2,4], Adrianna Frydrysiak-Brzozowska[2,5], Jargalsaikhan Badarch[2,6], Md Mozibur Rahman[2,7]

Received on: 20 January 2024; *Accepted on*: 18 February 2024; *Published on*: 26 March 2024

Abstract

Milk fat globules (MFGs) are a remarkable example of nature's ingenuity. Human milk (HM) carries contains 3–5% fat, 0.8–0.9% protein, 6.9–7.2% carbohydrate calculated as lactose, and 0.2% mineral constituents. Most of these nutrients are carried in these MFGs, which are composed of an energy-rich triacylglycerol (TAG) core surrounded by a triple membrane structure. The membrane contains polar lipids, specialized proteins, glycoproteins, and cholesterol. Each of these bioactive components serves important nutritional, immunological, neurological, and digestive functions. These MFGs are designed to release energy rapidly in the upper gastrointestinal tract and then persist for some time in the gut lumen so that the protective bioactive molecules are conveyed to the colon. These properties may shape the microbial colonization and innate immune properties of the developing gastrointestinal tract. Milk fat globules in milk from humans and ruminants may resemble in structure but there are considerable differences in size, profile, composition, and specific constituents. There are possibilities to not only enhance the nutritional composition in a goal-oriented fashion to correct specific deficiencies in the infant but also to use these fat globules as a nutraceutical in infants who require specific treatments. To mention a few, there might be possibilities in enhancing neurodevelopment, in defense against gastrointestinal and respiratory tract infections, improving insulin sensitivity, treating chronic inflammation, and altering plasma lipids. This review provides an overview of the composition, structure, and biological activities of the various components of the MFGs. 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: 1,4-β-N-acetylmuraminidase, Absorbable sphingosine, Acetyl-CoA carboxylase 1, Acyl-CoA synthetase, Acyl-CoA synthetase long chain family member 3, Acyl-CoA synthetase long chain family member 5, Adipophilin, Adipose differentiation-related proteins, ADPF, Alpha-1-antitrypsin, Annexin, Apocrine-like glands, Apolipoprotein A1, Apolipoprotein A-IV, Apolipoprotein C-III, Apolipoprotein E, Apolipoproteins, Arachidonic acid, Arginine-glycine-aspartate (RGD), *Bacteroidetes*, Bayley Scales of Infant and Toddler Development II, *Bifidobacterium*, Bile salt-stimulated lipase, Bone marrow stromal antigen 2, C16-ceramide, C18:0, C24-ceramide, Casein micelles, Cathelicidins, C-C motif chemokine ligand 2, CD9 antigen, Ceramidase, Ceramide, Ceramide-1-phosphate, Cerebrosides, *Chlorella vulgaris*, Cholesterol, Choline, Chordin-like protein 2,Clusterin, Complement C3, Conjugated linoleic acid, *Coriobacteriaceae*, De Brouckère mean diameter, Dermcidin, *Desulfovibrionaceae*, Diacylglycerol acyltransferase 1, Disialylated gangliosides, Docosahexaenoic acid, Elongase, Endoplasmic reticulum *Enterobacteriaceae*, *Enterococcaceae*, *Erysipelotrichaceae*, Exosomes, FA-binding protein, Factor V/VIII domain containing, Fagan test of infant intelligence, Fatty acid desaturase, Fatty acid synthase, Fatty acid-binding protein, *Firmicutes*, Folate receptor alpha, Food matrix, Free-play sustained attention test of Colombo, Gamma-glutamyltranspeptidase 1, Gangliosides, GD3, Gelsolin, Glutathione peroxidase 3, Glycam1, Glycan adhesion factors, Glycerol-3-phosphate acyltransferase 4, Glycobiome, Glycogen synthase kinase-3 β, Glycoproteins, Glycosphingolipids, Glycerol-3-phosphate acyltransferase 4, Glycogen synthase kinase-3 beta, Glycosylation-dependent cell adhesion molecule 1, Glycosylation-dependent cell adhesion molecule-1, GM3 Heat shock protein beta-1, Hormone-sensitive lipase, Human leukocyte antigen II, IgA α-chain, Insulin-like growth factor binding protein 2, Isobutyric acid, Isovaleric acid, Kyoto Encyclopedia of Genes and Genomes, Lactadherin, Lactating mammary gland packages, *Lactobacillus*, *Lactobacillus rhamnosus* GG, Lactoferrin, Lactophorin, Lactosome, Lanosterol synthase, Large-size MFG, Linoleic, Lipid rafts, Lipid-ordered microdomains, Lipoprotein lipase, Long-chain FA-CoA ligase, Lysophosphatidic acid acyltransferase, Lysozyme, Lysozyme C, MARCKS-related proteinapolipoprotein D, Mastitis, Matrilin-3, MFG epidermal growth factor 8, MFGE8 Microfiltration, Milk fat globule EGF, Milk fat globules, Monocyte differentiation CD14, Mucin 4, Mucins, *Mycoplasma agalactiae*, NAD(P) dependent steroid dehydrogenase-like, *Nannochloropsis gaditana*, Neutral glycosphingolipids, Nonspecific lipid transfer protein, Number-weighted mean, O-lined glycan, Pasteurization, Per-Arnt-Sim (PAS) domain 6/7, Perilipin, Perilipin 2, Peroxisomal acyl-coenzyme A oxidase 3, Peroxisomal bifunctional enzyme, Peroxisomal multifunctional enzyme type 2, Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol, Phosphatidylserine, Phospholipids, PL/TAG, Plasmalogens, Polar lipids, *Porphyromonadaceae*, Proactivator polypeptide, *Proteobacteria*, Proteose peptone component 3, *Rikenellaceae*, *Salmonella enteritides*, Sauter mean diameter, Small MFGs, Soluble *N*-ethylmaleimide–sensitive fusion attachment protein receptor, Sphingoids, Sphingolipid, Sphingolipids, Sphingomyelin, Sphingomyelin phosphodiesterase, Sphingophospholipids, Sphingosine, Sphingosine-1-phosphate, *Spirulina platensis*, *Staphylococcus aureus*, Triacylglycerol core, Tail-interacting protein-47, Tenascin, Toll-like receptor 2, Triacylglycerol, Triassic period, Ubiquitin, V/VIII like domains, Visual evoked potential latencies, Xanthine oxidoreductase, α-amylase, α-linolenic acid, αvβ3 integrin receptors, αvβ5 integrin receptors, β-casein, ζ-potential, ω-3 polyunsaturated fatty acids, ω-3 PUFA, ω-6 PUFA.

Newborn (2024): 10.5005/jp-journals-11002-0085

Key Points

- Human milk (HM) contains 3–5% fat, 0.8–0.9% protein, 6.9–7.2% carbohydrate calculated as lactose, and 0.2% mineral constituents. Most of these nutrients are carried in milk fat globules (MFGs).

[1]Department of Pediatrics, Louisiana State University, Shreveport, Louisiana, United States of America

[2]Global Newborn Society, Clarksville Maryland, United States of America

- The MFGs are composed of an energy-rich triacylglycerol core surrounded by a membrane structure. The membrane contains polar lipids, specialized proteins, glycoproteins, and cholesterol. Each of these bioactive components serves important nutritional, immunological, neurological, and digestive functions.
- Milk fat globules are designed to release energy rapidly in the upper gastrointestinal tract and then persist for some time in the gut lumen so that the protective bioactive molecules are conveyed to the colon. These properties may shape the microbial colonization and innate immune properties of the developing gastrointestinal tract.
- Understanding the composition and dynamics of MFGs could help enhance the nutritional composition of milk in a goal-oriented fashion.
- There is a possibility that MFGs could emerge as a nutraceutical for enteral delivery of medications in infants and older patients.

[3]Department of Physics, University of Illinois at Urbana-Champaign, Champaign, Illinois, United States of America

[4]Neonatology, Rainbow Children's Hospital and Birthright, Hyderabad, Telangana, India

[5]The Mazovian University in Płock, Collegium Medicum, Faculty of Health Sciences, Płock, Poland

[6]Department of Obstetrics, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia

[7]Neonatology, Institute of Child and Mother Health, Dhaka, Bangladesh

Corresponding Author: Akhil Maheshwari, Department of Pediatrics, Louisiana State University, Shreveport, Louisiana, United States of America, Phone: +7089108729, e-mail: Akhil@globalnewbornsociety.org

How to cite this article: Maheshwari A, Mantry H, Bagga N, *et al*. Milk Fat Globules: 2024 Updates. Newborn 2024;3(1):19–37.

Source of support: Nil

Conflict of interest: Dr Akhil Maheshwari is associated as the Editor-in-Chief of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of the Editor-in-Chief and his research group.

Introduction

Human milk is optimized for specific nutritional yield and bioactivities; there are complex lipids and proteins that serve important nutritional, digestive, immunological, and neurological functions.[1–4] Nearly 50% of the energy intake of HM-fed young infants comes from fat, which is equivalent to about 25 gm/day for about 6 months after birth.[5–7] The lactating mammary gland packages lipids in MFGs composed of a triacylglycerol (TAG) core with a surrounding triple membrane structure.[8–18] These globules contain polar lipids, specialized proteins, glycoproteins, and cholesterol.[16,19,20] These vesicles release energy rapidly in the upper gastrointestinal tract and then persist for some time in the gut lumen so that the protective bioactive molecules are conveyed to the colon.[1] These properties may shape the microbial colonization and innate immune properties of the developing gastrointestinal tract.[20] Milk fat globules in milk from humans and ruminants show some consistent structural features, but there are also notable differences in size, profile, composition, and specific constituents.[16]

In the last decade, several research groups have studied the organization and composition of MFGs.[3,4,14,20] Milk fat globules in human and ruminant milk share the basic structure but show differences in the concentration and proportions of polar lipids and the profile of proteins.[16] Some beneficial components of the MFG membrane such as lactadherin, ω-3 polyunsaturated fatty acids (FAs), and phospholipids are enriched in specific size fractions of the MFG pool.[16,21–23] The size and composition of MFGs change with the stage of lactation, ethnicity, and maternal diet.[1,16,24] This review summarizes our current understanding of MFGs and related bioactive components. Our primary focus is on HM but when needed, we have included information from studies of other ruminants.

Milk fat globules in HM vary in size, physical properties, fractions, and composition.[16] This information can be potentially important for: (a) our understanding of the actual bioavailability of measurable nutrients in HM;[20] (b) improving our understanding of the need for specific nutrients, vis-à-vis the constituents of MFGs, to design better strategies for treatment of intra- and/or extra-uterine growth restriction;[25] (c) to determine the impact of high concentrations of MFGs in low-volume, calorie-dense feedings that would not pose risks due to hyperosmolality in very premature or critically-ill infants;[20] (d) a la fractionation of blood meant for transfusion into specific components such as packed cells, plasma, and other components, identification and isolation of specific MFG subclasses and soluble whey nutrients to improve targeted, efficient use of banked HM;[11] (e) designing strategies to prevent contamination in different fractions;[26] (f) once the temporal deterioration of various fractions is understood, designing specific storage strategies;[27] (g) designing strategies for optimal transportation of milk fractions in warmer peri-equatorial and tropical regions;[28] (h) reducing the risk of transmission of infectious diseases;[29] (i) retrieval of some "contaminated" milk components by specifically designed pasteurization, filtration, and/or other procedures;[30] and finally, (j) the possibility of engineering artificial MFGs for enteral delivery of nutrients, vaccines, and/or medications in critically-ill infants.[31] Such products could also help in 'humanizing' MFGs derived from other mammals that have important biological differences, or for complete de novo manufacturing for specifically-designed therapeutic vehicles.[27,32] These products could also be useful for infant nutrition in societies with religious/social restrictions on the use of banked HM.[33]

Milk Fat Globules (MFGs)

Dimensions

Milk Fat Globules are typically sized between 0.1 and 15 μm in diameter.[34] The globules contain a nonpolar TAG core covered by a surface-active membrane; these structural characteristics stabilize these globules in the emulsion and prevent enzymatic degradation and coalescence.[1] Milk lipid droplets measuring <0.5 μm are first seen in the rough ER and are then coated with phospholipid monolayers before translocation to the apical plasma membrane.[35] Many of these smaller droplets fuse together to gain size and then get additional lipid coatings from some components of the plasma membrane.[31] In HM, most MFGs measure about 4.2–5.1 μm in diameter;[16] the size (range distribution and average) of these globules is determined by factors such as including genetics, maternal diet, duration of lactation, and may also be altered to fit the nutritional requirements of the infant.[11]

The size of MFGs can be described by diameter, volume, number, and surface area.[16] The mean diameters of MFGs are commonly expressed as a number-weighted mean (d_n, $D_{1,0}$), volume-weighted mean (d_{vm}, $D_{4,3}$), or as the surface-weighted mean (d_{vs}, $D_{3,2}$).[16] The surface-weighted mean (Sauter mean diameter) emphasizes

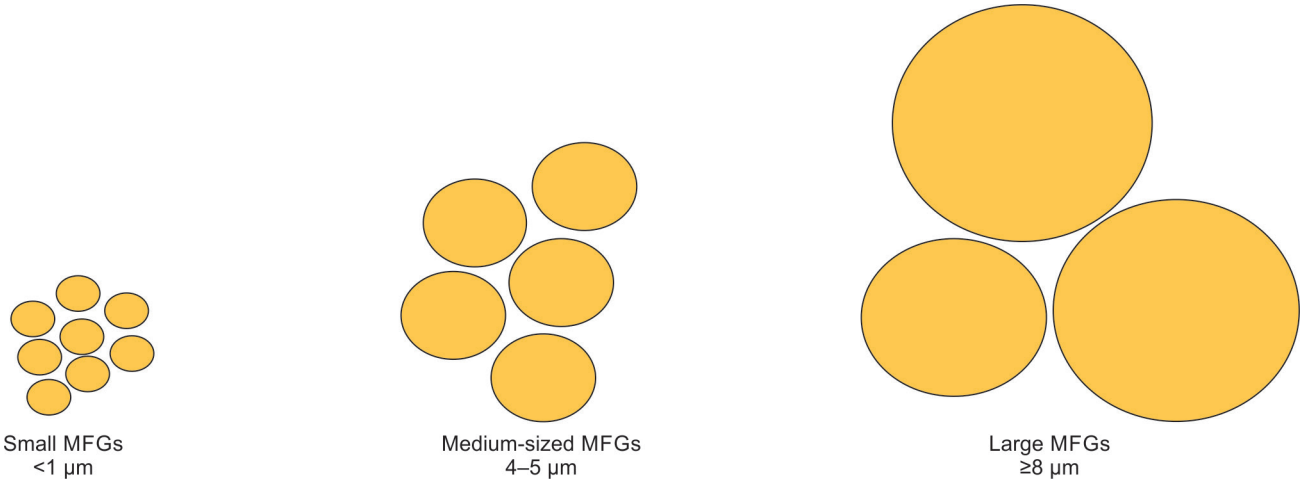


Fig. 1: Milk fat globules are typically sized between 0.1 and 15 µm in diameter. There are 3 important volumetric subgroups, including the small (<1 µm diameter), medium (4–5 µm), and large (≥8 µm)

the globule surface area, as it is an important determinant of phenomena such as the release of components from surfaces, 2-dimensional surface reactions, and surface dissolution.[36,37] The size distribution by volume varies as smaller globules have a higher specific surface area: volume ratio compared to larger globules. The volume-weighed mean (De Brouckère mean diameter, d_{vm}, $D_{4,3}$) reflects the contribution of each particle in the distribution related to the volume.[38,39] Colostrum contains some of the largest MGFs. The size then decreases somewhat in transitional milk, and then it increases again by 7–10 days after birth.[17] Milk fat globules have been typically classified in three 3 volumetric subgroups (Fig. 1):

1. Small MFGs (<1 µm in diameter), which comprise nearly 80% of all globules and form a very large lipid interface surface, which is the key parameter in determining the susceptibility of MFG membrane to digestive hydrolysis and reactivity.[16] These particles contain unsaturated FAs, and may have additional functional effects on lactation beyond carrying and emulsifying the TAG core;[20]
2. Medium-size MFGs; these carry most (nearly 95%) of the volume of the lipid phase;[15]
3. Large-size MFG (≥8 µm in diameter), which typically form from fusion of medium-size MFGs.[15,16]

Milk fat globule size varies during different stages of lactation.[16] These variations likely optimize maternal energy costs to supply nutritional and bioactive components. Milk fat globules in human colostrum (≤4 days after delivery) are typically larger than those in transitional and mature milk.[16] Large-sized globules seen during early lactation could well be related to the immaturity of mammary glands, related to the inability to produce membrane phospholipids at the same pace at TAGs.[20] In contrast to the diameter, the average surface area of MFG increased from 1.1 ± 0 to 5.4 ± 0.7 m^2/g during the transition of colostrum to mature milk but the volume-weighted mean decreased.[40] Even though some variation is seen in the size of MFGs during lactation, the average diameters of mature MFGs remain relatively stable at around 4.5 µm.[16] The size changes are directly correlated to changing lipid and protein constituents.[16]

Composition

Human milk contains 2.8–3.8 gm/100 gm fat and phospholipids but these concentrations differ by species.[3,41] Ovine milk contains 6.5–9 gm/100 gm, caprine 3.5–5.6 gm/100 gm), and bovine 3.4–6.0 gm/100 gm. The most important lipids in MFGs are TAGs (98%), polar lipids, and cholesterol;[42] the TAG fraction is the predominant source of energy.[11]

The fat content of milk changes with the maternal environment, diet, and physiological state.[43] Colostrum contains more total phospholipids, stearic acid, long-chain polyunsaturated FAs, and gangliosides than transitional and mature milk.[44] FAs with 4–14 carbons can be synthesized *de novo* in the mammary gland, whereas the 16-carbon (16C) FAs are usually derived from maternal diet, circulation, or her own body fat stores.[3] As milk matures, increased production of 12–14C medium-chain FAs is reflected as decreased average length of the FA chains. Long-chain FA content remains similar throughout lactation. Triacylglycerol levels increase in the first few weeks after birth, but those of cholesterol and cholesterol esters gradually decrease. Sphingomyelin levels remain stable.

There is a gradual shift from the disialylated gangliosides (GDs), particularly GD3 (disialosylganglioside; Neu5Aca2-8Neu5Aca2-3Galb1-4Glc-Cer) in colostrum to GM3 (monosialodihexosylganglioside; alpha-Neu5Ac-(2->3)-beta-D-Gal-(1->4)-beta-D-Glc-(1<->1')-Cer(d18:1/18:0) in mature milk.[45] Colostrum gangliosides contain more long chain- and less medium-chain FAs than those in mature milk. To recall, gangliosides are classified according to the number of sialic-acid residues on the molecular backbone (M = mono- or 1; D = di- or 2, as GM or GD), the number of residues attached to the sugar moiety, and the biosynthetic pathway from which these are derived.[46] Similarly, there are more monounsaturated FAs than long-chain FAs.[20] In the following sections, we present current data for fat/protein fractions in MFGs.

Fats in MFGs

Small MFGs contain more dietary FAs than the larger ones, although the proportion of *de novo* synthesized FAs may not be different.[17] Shorter (4–16C) FAs incorporated into these globules are typically obtained from de novo synthesis, whereas the longer ones (≥16C) are derived from TAGs in diet or adipose tissue.[17,47] During early lactation, low phospholipid/TAG ratios in the membranes and longer FAs (C17:0, C18:0, and C20:0) can enhance large globule formation.[17] Low PE levels and high PC: PE ratios can make MFGs less amenable to fusion because of lower interfacial surface tension.[48]

Mucin–1 and –15 and FAs such as C10:1, C11, C12, C12:1, C13:0, CD14:0, and C15:0 also increase the formation of small MFGs.[16] For each unit of fat, small MFGs present a larger total surface area for lipase action, and consequently, get digested faster.[49] There are also differences in the interaction of globules of different sizes with specific enzymes.[16]

Milk fat globule size is determined primarily by the phospholipid/TAG ratio, FA composition, and cholesterol content.[20] Smaller globules may contain more phospholipids.[9] Higher intracellular PE content can promote droplet fusion to produce larger MFGs.[18] In bovine milk, the type of esterified FAs in the lipids in the MFG core and membrane may also alter the size of the globules; longer long- and medium-chain FAs may lead to smaller MFGs.[20] Small and medium-sized MFGs may also show different digestion and fat release patterns.[20] GM (monosialylated GAs) and GD (disialylated GAs) are the two major GA species in HM, and GD_3 (Neu5Aca2-8Neu5Aca2-3Galb1-4Glc-Cer) is the most abundant GD in HM.[11]

In cattle, a higher intake of saturated lipids (50% palmitic acid) resulted in higher fat content in milk with larger MFGs.[50] In contrast, high polyunsaturated FA intake reduced MFG size with proportionately more polar lipids and unsaturated FAs. Linoleic (LA) (C18:2c9,12), α-linolenic (ALA) (C18:3c9,12,15) and palmitic (C16:0) acids accounted for approximately 88% of total FAs.[51] Dietary supplements of conjugated linoleic acid (*cis*-11, trans-9 and trans-10, cis-12 isomers) also suppressed milk fat synthesis with decreased MFG diameter.[51]

Membrane phospholipids represent only 0.5-1% of the total fat in milk but contain 15–20% of the total LC-PUFAs in milk.[20] In contrast, membrane SLs are highly saturated and maintain the lipid rafts, which might facilitate the delivery of sphingosine and ceramides to the distal gastrointestinal tract.[20] Glycosphingolipids, such as cerebrosides and gangliosides, are present in relatively low concentrations. Cerebrosides are neutral glycosphingolipids containing uncharged sugars.[52] In bovine milk, lipid metabolism may regulate the production of small or large MFGs. Small MFGs (average 3.29 μm) contained higher concentrations of unsaturated FAs compared to larger globules (average 4.92 μm). These findings could have been related to higher uptake of long-chain FA from the blood circulation in smaller MFGs.[17]

Colostrum contains more medium- and poly-unsaturated FAs than mature milk.[53] C18:1 ω-9 seems to be the most abundant FA in colostrum and milk, followed by C16:0, C18:0, and C18:2 ω-6. C18:1 ω-9 concentrations decreased, whereas C:16 increased from colostrum to milk. In general, smaller MFGs (<L3 μm) contain more medium-chain FAs (C10:1, C11, C12, C12:1, C13) such as myristic (C14:0) and pentadecanoic acid (C15:0) and a larger fraction of total unsaturated FAs. Large MFGs contain more long-chain saturated FAs (C17:0, C18:0, and C20:0).

Proteins in MFGs

Proteins are an important determinant of MFG size; the relative content of xanthine oxidase/xanthine oxidoreductase (XO/XOR) and butyrophilin (BTN)-1 is an important variable.[12] During early lactation, α-amylase, MARCKS (myristoylated alanine-rich C-kinase substrate)-related apolipoprotein D, apolipoprotein E, ubiquitin, bone marrow stromal antigen 2, chordin-like protein 2, gamma-glutamyltranspeptidase 1, long-chain FA-CoA ligase 4, α-1-antitrypsin, insulin-like growth factor binding protein (IGFBP)-2 and matrilin-3 show higher expression. In later stages, annexin, complement C3, CD9 antigen, nonspecific lipid transfer protein, FA-binding protein, folate receptor alpha, glutathione peroxidase 3, gelsolin, heat shock protein beta-1, lysozyme C, proactivator polypeptide, BTN and XOR are more highly expressed.[16]

Milk fat globule size is an important predictor of protein signatures.[54,55] Small MFGs typically contain proteins needed for lipid metabolisms such as acyl-CoA synthetase long-chain family member 5 (ACSL5), acyl-CoA synthetase long-chain family member 3 (ACSL3), and glycogen synthase kinase-3 beta (GSK3B), lanosterol synthase (LSS), acetyl-CoA carboxylase 1 (ACC), NAD(P) dependent steroid dehydrogenase-like (NSDHL), and glycerol-3-phosphate acyltransferase 4 (GPAT4). Larger globules contain more lactadherin (gene Milk fat globule EGF, factor V/VIII domain containing; MFGE8), adipophilin (adipose differentiation-related protein, also known as perilipin 2, adipophilin; gene ADPF), and the ratio of glycam1 (glycosylation-dependent cell adhesion molecule 1)/lactophorin (gene Proteose peptone component 3; PP3) than smaller ones. Size might also alter surface polarity, and consequently, the FA and phospholipid composition. Differences in protein composition might be a secondary change throughout lactation.

Impact of processing of milk: The effect of the processing of HM on MFG structure needs further investigation. Some studies suggest that MFG FAs and TAGs may not be affected by homogenization and thermal processing, whereas others, working with bovine milk, have shown conflicting data.[28,56–58] Freezing of HM may result in the loss of some fat and of the MFG structure.[1] Some FAs formed during cold storage of HM may be cytotoxic.[1] Small vs large MFGs may be differentially affected by digestion due to variations in lipid profiles.[59] In bovine milk, smaller MFGs contain less long-chain but more medium-chain FAs and myristic acid in the core.[47] Gastric lipases preferentially hydrolyze short- and medium-chain FAs, and so the size of fat globules will affect the digestion rate and release of these FAs.[60] The profile and concentrations of phospholipids may also affect lipid absorption and metabolism.[61]

Homogenized MFGs can be digested more rapidly than native globules, although more details are needed on changes in surface area.[8] Homogenization can promote the binding of milk proteins to the MFG membrane, which avidly binds pepsin and pancreatic lipase.[11] However, MFG membrane proteins such as BTN, pulmonary adenoma susceptibility (PAS)-6/7, and mucins are resistant to proteases.[62]

Microfiltration is a useful way to purify MFGs with specific sizes.[27,63] Hansen et al.[64] have isolated MFG membranes containing 7% w/w polar lipids and 30% w/w proteins. They used ceramic dia-microfiltration of raw whole milk to first separate fat globules from casein micelles and whey proteins, and then started the procedures for MFG membrane extraction. Pasteurization (72°C, 15 seconds) prior to or after microfiltration had no impact on filtration efficiency or on MFG membrane yield and composition.[17]

Human milk also contains other extracellular lipid-enclosed structures such as exosomes and casein micelles (100–200 nm in diameter).[16,65] These subgroups can be dissociated using EDTA during milk processing, and so the presence of exosomes may confound the measurements of MFG size based on light-scattering analysis. There are also some smaller 25–30 nm lactosome particles, which do not contain a TAG core.[66] The lactosomes do not show major morphological changes over time. These particles contain osteopontin and β_2-microglobulin, which are involved in early innate and adaptive immune responses. However, there are no gangliosides, which would have enabled interaction with many bacterial toxins. In the context of nutrition, these particles contain α-lactalbumin, which is a component of lactose synthase, and

Eon	Era	Period	Millions of years ago
	Cenozoic	Quanternary	1.6
		Tertiary	66
	Mesozoic	Cretaceous	138
		Jurassic	205
		Triassic	240
Phanerozoic	Paleozoic	Permian	290
		Pennsylvanian	330
		Mississippian	360
		Devonian	410
		Silurian	435
		Ordovician	500
		Cambrian	570
Proterozoic			
Archean			
Hadean			

Fig. 2: Evolution of milk: Some therapsids and Mammalia-formes began producing a milk-like secretion during the Triassic period 220–280 million years ago (indicated by the red line)

phospholipids. However, the absence of a TAG core excludes the possibility of a major role in energy delivery. As currently understood, lactosomes do not share secretory pathways with MFGs.

Endocrine Regulation of MFG Size

Hormones such as prolactin and oxytocin can alter MFG size.[67] In bovine milk, progesterone is also an important regulator of MFG size.[68] Lipid droplets smaller than 3 µm may be more abundant in the luteal phase with higher progesterone levels than in the follicular phase.[66] The effect of progesterone was mediated via long-chain FAs in the mammary epithelium. The details of these findings still need to be elucidated.

High plasma insulin concentrations can lower the TAG: phospholipids ratios in the MFG membrane and raise the concentrations of monounsaturated FAs in the MFG core.[16] Negative systemic energy balance during the early stages of lactation can also alter MFG size.[69] MFGs produced during this period may contain more oleic acid (C18:1 *cis*-9), less palmitic acid (C16:0), an overall reduction in *de novo* synthesis of FAs, and a reduction in the size of MFGs.[16]

Ontogeny and Phylogeny of MFGs

Lactation appeared as a reproductive feature prior to the origin of mammals.[70,71] Mammals gradually accrued from synapsid ancestors, and the mammary gland may have evolved from apocrine-like glands, which were associated with hair follicles and provided moisture and antimicrobials to parchment-shelled eggs.[72–74] Some therapsids and the mammalia-formes began producing a nutrient-rich milk-like secretion during the Triassic period 220-280 million years ago (Fig. 2).[75,76] Genes encoding for MFG membrane proteins in milk are highly conserved, particularly those with nutritional or immunological attributes.[20,70] This mechanism is important for milk-fat secretion. Milk fat globule seem to have evolved by co-opting membrane (BTN), cytosol (XOR), and intracellular lipid droplet (adipophilin) proteins into new/expanded functions.[77–80]

Biophysical Forces Acting on MFGs

Liquid surfaces incur a significant energy cost as a large number of intermolecular bonds need to be broken to create a surface.[81] Consequently, the MFG droplets evolve into spheres over time to minimize the surface area.[82] Furthermore, smaller droplets aggregate into larger ones; a process that has been named as flocculation.[83] If we consider a fat globule with a radius *r*, the surface energy (*U*) of the droplet could be calculated as the product of its total surface area ($4\pi r^2$) and the surface tension (γ_f); $U = 4\pi r^2 \times \gamma_f$.[84] If the square of the radius length determines the surface energy, a sphere would accord the least surface area of all 3-dimensional structures, and hence, carry the least surface energy. If two smaller droplets were to coalesce into a single larger droplet, the radius of this larger particle would be $R = 2^{1/3} \times r$. The total surface energy of the initial smaller droplet would be $U_1 = 8\ \gamma_f \times \pi r^2$, but that of a larger droplet would be lesser ($U_2 = \gamma_f \times 4\pi r^2 = 2^{2/3} \times 4\pi r^2$). As every natural system seeks the lowest energy state, such aggregations would be favored.[85]

Another observation of MFGs needs discussion. These globules show a continuous jiggling motion, which might stabilize these particles.[86] Current understanding ascribes this phenomenon to the Brownian motion of water molecules but this might be an oversimplification; a number of disparate forces might actually be

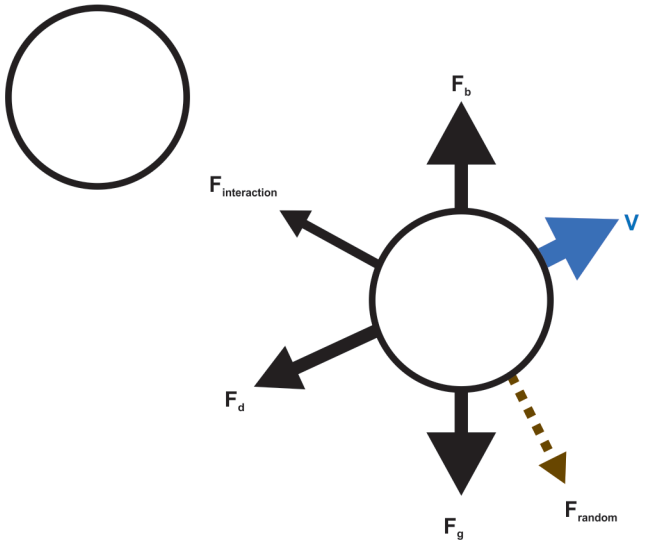


Fig. 3: Forces acting on MFGs likely include (clockwise): (A) Gravitational weight (F_g); (B) Drag force (F_b); (C) $F_{interaction}$, a summated effect of the van der Waals and the electrostatic forces. These interactions could be attractive or repulsive depending on the intrinsic characteristics of the globules and the separating distance. As depicted above, the $F_{interaction}$ forces should be visualized along a line joining the centers of the two spheres; (D) Buoyant force (F_b); and (E) Random forces (F_{random}). V refers to the velocity of the Brownian particle

involved here.[87–89] Further work is needed to understand these factors because deep freezing, the current standard practice for preserving maternal milk for later use, and pasteurization, used for sterilization, may alter the stability of MFGs.

In a highly-idealized model, the MFGs would be subjected to the following forces (Fig. 3):

- Gravitational weight, $m \times g$, a product of m, the mass of the particle, and g, the local acceleration due to gravity;[90]
- Buoyant force exerted by the solvent, the Archimedes principle, which would be water in the aqueous base of milk.[89] This would be computed as a product of the density of water, ρ_w, V as the volume of volume of the particle, and g as the local acceleration due to gravity; $F = \rho_w \times V \times g$;
- Drag force, a viscous force described by Stokes law.[91,92] This would depend on the speed (v) and the radius of the particle (r), and the constant of viscosity (η); $D = 6\pi \times \eta \times r \times v$;
- Random forces due to the thermal fluctuation, which would augment the Brownian motion of solvent molecules.[93] These movements might, paradoxically, stabilize the MFGs;
- Electrostatic forces between the charged phospholipid surface layers, the polar solvent, and other globules;[94]
- van der Waals forces between the organic part of the particles.[95,96]

Further work is also needed to understand Brownian motion[97] of MFGs. Despite the relatively large size of MFGs, these might still simulate ideal gas/Brownian particles.[98] The velocity scale can be viewed as $\sqrt{(k_B \times T/m)}$, where k_B would be the Boltzmann constant, T the temperature, and m the mass of the Brownian particle.[99–101] In this equation, the characteristic time scale of motion or the time needed by a particle to re-attain the state of rest after a collision would be $m/(6\pi \times \eta \times r) \propto r^2$. If this relationship stands correct for MFGs, smaller particles should attain a state of rest faster than larger ones and are therefore likely to be more stable. Smaller particles may also show longer intervals between two successive collisions. However, there are numerous assumptions in these models and further work is needed for this line of investigation.

Finally, there also have been discussions on developing mathematical models to understand the behavior of MFGs as single units. A relationship between membrane tension and gravity could help understand the spreading of membrane-enclosed vesicles, and consequently, membrane tension-related biological processes. Wang et al.[90] showed that equilibrium differential equations could be one plausible way to relate forces such as gravity, internal pressure, and membrane tension: (a) the deformed geometry in the vesicle models could be represented by a pseudo-ellipsoidal or pseudo-spherical cap under the action of gravity;[102] the pseudo-ellipsoidal cap may be more plausible from a mathematical point of view; (b) the membrane tension may decrease with distance from the basal surfaces; (c) the inclination between a tangent and a radial line might correlate with the locally-defined principle radius; (d) gravity could be an important variable in the spreading of vesicles as it might influence the distribution of membrane tension. These efforts may help in predicting the effects of gravity on the deformation of vesicles.

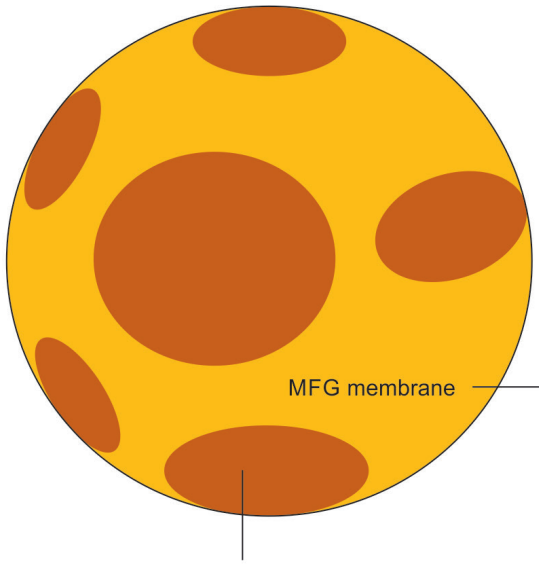
Milk Fat Globule Membrane

Structural Characteristics of the MFG Membrane

Milk Fat Globules are covered with a continuous, 10–50 nm (usually 15–20 nm) membrane derived from the cytoplasm following secretion into the alveolus.[16,17] It accounts for about 6% of the globular mass, and is constituted of about 60% proteins and 40% lipids. It stabilizes the globules in the emulsion.[16,17]

There is some evidence to show that the MFG membrane may include two distinct lipid phases. The first may be comprised of relatively less dense, liquid-disordered regions with unsaturated glycerophospholipids, proteins, glycoproteins, glycolipids, and some SM.[20] The second may show a distinct biphasic separation of SM and cholesterol into densely-packed, liquid-ordered domains called the 'rafts' (Fig. 4).[103,104] In these microdomains, SM interacts asymmetrically with large amounts of cholesterol in circular assemblies. Sphingomyelin and glycerophospholipids differ in head group structure, hydrocarbon tail length, and degree of unsaturation.[20] There may well be other types of microdomains enriched in other glycerophospholipids.[48]

Traditional models of the MFG suggest a 3-layered membrane covering of the TAG-rich core (Fig. 5).[11,18,20,34,62,63,105,106] The innermost is a surface-active, ER-derived layer comprised of polar glycerophospholipids such as PE, PI, and PS; proteins; SM; and gangliosides. The middle is relatively dense and rich in proteins. The outer two layers are rich in polar lipids, which are translocated across the apical plasma membrane of mammary epithelial cells. Many phospholipids and SM are located on the external aspect of the membrane. Some of the glycoproteins and glycolipids are also loosely attached to the surface, where the carbohydrate domains project into the surrounding aqueous phase. These have antimicrobial, anti-inflammatory, and prebiotic functions in the gut. In addition, there are many transmembrane proteins and cholesterol molecules. The distribution, size, and profile of polar lipids and proteins in the MFG membrane vary with diet and among species.



Glycerophospholipids such as phosphatidylethanolamine, phosphatidylcholine phosphatidylinositol, phosphatidylserine

Glycolipids such as gangliosides

Fatty acids (C10:1, C11, C12, C12:1, C13:0, CD14:0, C15:0, C16:0, C17:0, C18:0, and C20:0
ω-3 and ω-6 PUFAs

Phospholipids

Gangliosides, GM (mono-sialic) and GD (di-sialic)

Proteins such as butyrophilin (BTN), xanthine dehydrogenase/oxidase (XDH/XO), adipophilin (ADPF; perilipin-2, PLIN2), mucins-1 and -15 stomatin, MFG epidermal growth factor 8 (MFGE8, lactadherin), glycosylation-dependent cell adhesion molecule-1 (GLYCAM1), proteose peptone component 3 (PP3), α-lactalbumin, lysozyme, β-casein, clusterin, lactoferrin, IgA α-chain, tenascin, apolipoprotein A-I, FA synthase

Sphingomyelin (SM) 'rafts': Microdomains containing SM and its metabolites such as ceramide, sphingosine, ceramide-1-phosphate, and sphingosine-1-phosphate

Cholesterol

Other kinds rich in glycosphingolipids such as cerebrosides and gangliosides

Fig. 4: The MFG membrane may include two distinct lipid phases. The first set may be comprised of relatively less dense, liquid-disordered regions with unsaturated glycerophospholipids, proteins, glycoproteins, glycolipids, and some SM. In the second, SM and cholesterol form densely-packed, liquid-ordered domains, the 'rafts'

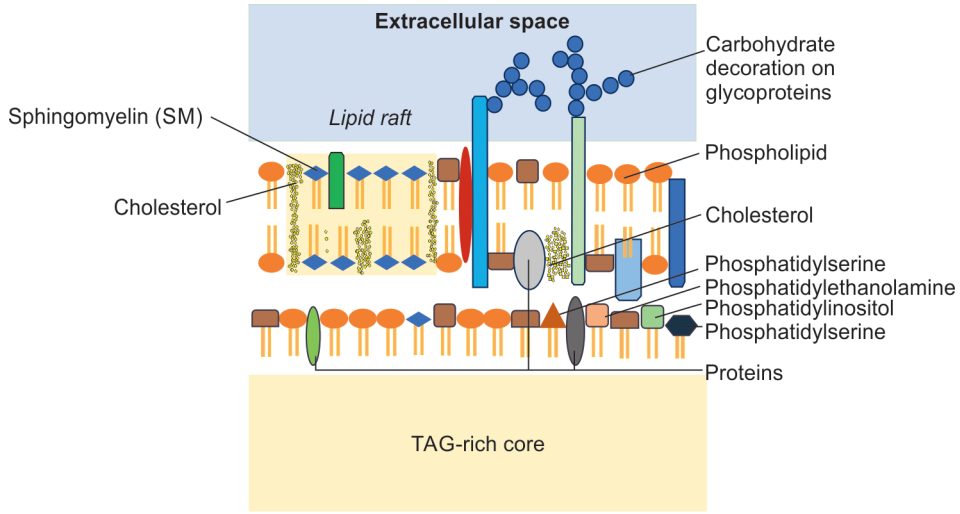


Fig. 5: The MFG membrane is a triple-layered membrane, where the external two layers are derived from the apical membrane. The proteins extending from the surface are heavily decorated with carbohydrates. Most of the MFG membrane shows a liquid-ordered phase with SM in close association with cholesterol. Some microdomains, the lipid rafts, are relatively rigid and play an important role in cell signaling. The internal layer is a loosely-packed glycerophospholipid matrix containing unsaturated glycerophospholipids such as phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine. This layer is derived from the endoplasmic reticulum and typically contains two unsaturated FAs bound to a glycerol backbone

Some of the cholesterol in the globular membrane might interact with other glycerophospholipids in the liquid-ordered domain and protrude from the liquid-disordered domain. Sphingomyelin and glycerophospholipids differ in structural characteristics in terms of head group structure, hydrocarbon tail length, and degree of saturation.[11] Sphingomyelin (SMs) show asymmetric molecular structures and high hydrogen-bonding potential, which influence the stability of MFGs.[107]

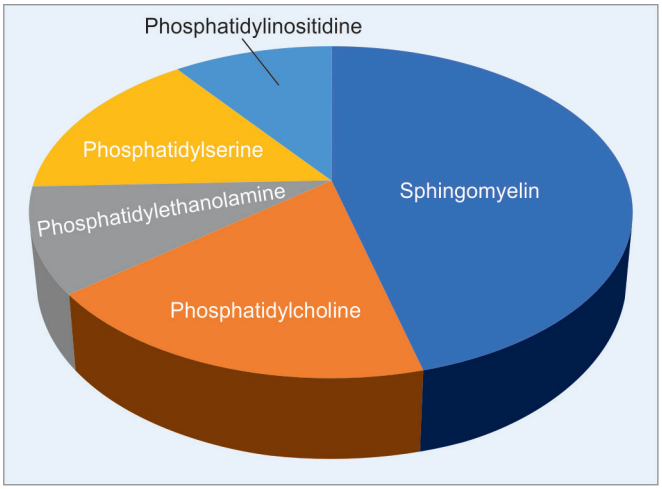


Fig. 6: Milk fat globules contain polar lipids. The most important constituents include sphingolipids such as sphingomyelin, and glycerophospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and phosphatidylinositidine. Sphingomyelin is present in higher quantities in HM than in milk from other mammals

Lipids in the MFG membrane: Milk fat globule membranes typically contain TAGs (56–62%), polar lipids (26–46%), and some minor constituents such as diacylglycerols, free fatty acids (FFA), and sterols.[108] This hydrophobic core is covered by a protein-rich hydrophilic phospholipid membrane, which contains about 40% lipids and 60% proteins.[108] During passage through the ER and the Golgi to the apical plasma membrane, these droplets fuse together and grow. Whey proteins, casein, and lactose are added to the Golgi apparatus. The budding lipid droplets get covered with an electron-clouded inner face of the plasma membrane, which eventually forms the lipid bilayer of the MFG membrane.[109] The extrusion from mammary epithelial cells is mediated by FA transporters, such as the FA binding protein (FABP), acyl-CoA binding protein, and FA translocator CD36.[110,111] Gangliosides play a key regulatory role in this process.[20]

Polar lipids: Milk fat globule membranes contain many polar lipids, which vary by the size of MFGs, maternal ethnicity and geographical origin, and her diet.[17] The most important polar lipids include sphingolipids such as SM, and glycerophospholipids such as PE, PC, PS, and PI (Fig. 6). Sphingomyelin is present in higher quantity in HM than in milk from other mammals.[49] PE, PC and SM are the most abundant phospholipids in the MFG membrane; each constitutes about 30% each of the total phospholipid content in the MFG membrane. PS and PI contribute 5–10% each.

Compared to whole milk and large MFG fractions, the small MFG fractions are relatively enriched in polar lipids.[105] The phospholipid: TAG ratios in the membrane can also affect MFG formation. Low phospholipid/TAG ratios enhance large MFG formation.[20] High PE concentrations can promote droplet fusion by lowering the interfacial surface tension, but high PC: PE ratios can inhibit droplet fusion. Overall, HM contains less PE than in ruminants.[11] Mucins 1 and 15, high PL/TAG ratios, and certain FAs (C10:1, C11, C12, C12:1, C13:0, CD14:0, and C15:0) can increase small MFG formation. Similarly, C17:0, C18:0, and C20:0 FAs can also augment this process.[16,17,112]

Postpartum changes in the phospholipid content of milk need further study.[113] The total phospholipid concentrations and MFG size show considerable variability during the neonatal period.[3,114,115] On the day of delivery, most globules might be ≥10 µm in size and then become smaller rapidly to ≤1 µm by day 4. This correlates with increased total phospholipid and fat concentrations from colostrum to mature milk. However, PC, PI, PE, and PS concentrations may not always show a consistent pattern during the transition from colostrum to mature milk. Overall, the membranes of small globules contain lower concentrations of PC and SM.[20]

Milk phospholipids have been shown to improve cognition, neuroplasticity, and myelinization in both animal models and clinical studies.[116] In term infants, MFG membrane supplementation begun in the 1st week seems safe.[117] We still need to study preterm infants, who may possibly show greater benefit because of lower stores. HM and MFG membrane components may possibly protect against necrotizing enterocolitis, stunted brain growth, and brain injury, retinopathy of prematurity, and infections.[10,118] The impact on eczema is uncertain.[118]

Fatty acids (FAs): Human MFG membranes contain more unsaturated FAs than the core TAGs.[20] Small MFGs may contain more unsaturated FAs because of the proportionately higher content of membrane material than in large globules. C16:0 is the most abundant FA in colostrum and early milk, and the levels decrease progressively with time. The opposite trend was seen in C18:0, ω-3 PUFAs, and ω-6 PUFA levels.[119] Unlike HM, infant formulae contain plant oil-based lipids that lack some of these components.[120] Milk fat globule membranes contain about 15% of the long chain-polyunsaturated FAs (LC-PUFAs), most of which are bound to phospholipids.[20] Compared to the total lipid compartment, phospholipids contain relatively more stearic acid but less oleic acid.[52] Except for PC, palmitic acid also constitutes a smaller proportion of milk phospholipids. There is a high arachidonic acid (ARA; 12%) content in PE and PI. Docosahexaenoic acid (DHA) contributes up to 5% and 3% in PE and PS, respectively.[121] In contrast, the SM content (0.4%) resembles that of total lipids.[43] Although the bioavailability of TAG and phospholipid-bound LC-PUFA might not differ, the metabolic disposition including the incorporation into the brain might be affected.[122] Thus, even though the nutritional importance of the MFG membrane lipids may not be affected by the LC-PUFA content, these are important as a source of specific lipids and a role in the development of various organs.

Gangliosides: Both endogenous and dietary GM3 and GD3 gangliosides activate the mucosal immune system by increasing the production of cytokines and IgA, and lymphocyte function.[10,123,124] In contrast, these gangliosides inhibit dendritic cells and could possibly promote tolerance against non-aggressive antigens.[125] Overall, HM gangliosides are believed to promote infant gut maturation with effects on neuronal growth, migration, maturation, neuritogenesis, synaptogenesis, and myelination.[126] HM contains high levels of gangliosides right from birth, and could improve cognitive development in infants aged 0–6 months.[45,127]

The MFG membrane is an exclusive carrier of gangliosides, particularly GD3 to the neonatal gut.[128] Gangliosides get incorporated into the intestinal mucosa and alter membrane fluidity and enterocyte function.[129] These are integral components in cell membranes, and the oligosaccharide residues that extend from the cell surface promote cell-cell communication. Dietary gangliosides can increase ether phospholipids and the uptake of LC-PUFAs.[130]

Sphingomyelin (SM): Sphingomyelin accounts for 25% of the total milk polar lipids and is complexed with cholesterol in a mass ratio of 3:1.[131] HM-fed infants obtain about 150 mg SM per day.[11]

Sphingolipids are built on a backbone of sphingoids, a set of aliphatic amino alcohols.[132] In the MFG membrane, SM is important as it is the most important constituent of sphingolipids along with glucosyl- and lactosylceramides.[49,114]

In neonates, orally-ingested intact SM is not absorbed but it may still accelerate gut maturation.[115] The alkaline sphingomyelinase and ceramidase expressed on gut epithelium convert sphingomyelin to absorbable sphingosine, which can then be converted to sphingosine-1-phosphate. Sphingosine can inhibit protein kinase c, and induce cell cycle arrest and apoptosis.[133] In the intestine, sphingosine-1-phosphate may activate inflammation, angiogenesis, vascular permeability, and organ development.[134] The SM: cholesterol ratio in the MFG membrane can alter its structure with changes in temperature during cooling, storage, heating, and digestion.[135]

Sphingomyelin concentrations in HM remain largely unchanged over time in the postnatal period.[136] Its metabolites, including ceramide, sphingosine, ceramide-1-phosphate, and sphingosine-1-phosphate are important in inflammation and cell differentiation/apoptosis.[137] The clinical effects of SM are still being studied; in a pilot study, 24 very-low-birth-weight infants were randomized to receive HM with added standard milk (SM 13% of all phospholipids) or SM-fortified milk (SM 20% of all phospholipids).[138] Neurodevelopmental follow-up between 6-18 months after birth showed that SM-fortification improved behavior rating on Bayley Scales of Infant and Toddler Development II, the Fagan test of infant intelligence, visual evoked potential latencies, and free-play sustained attention test of Colombo.[138]

Choline: Choline is a highly methylated component of membrane constituents, PC, SM, and choline plasmalogens; and of the neurotransmitter acetylcholine.[139] It may be measurable as its unesterified form, or as phosphocholine, glycerophosphocholine, PC, and/or SM. PC and SM in the MFG membranes contribute about 10% to the total choline intake of infants.

The fetus and the neonate have high levels of choline in the blood and tissues as it is important in neurodevelopment.[140] Low choline status in early pregnancy increased the risk of neural tube defects and poor cognitive development. It is also important for neurogenesis and synaptogenesis; dietary supplementation can potentially promote cognitive functions and overall infant development. In one study of bovine colostrum and milk, the concentration of 26 MFG membrane proteins increased and 19 decreased at 7 days of lactation.[16] The concentrations of mucin-1 and –15 increased 7-fold, ADPF 3.4-fold, BTN 3.2-fold, and XDH 2.6-fold. The concentrations of acyl-CoA synthetase, lanosterol synthase, lysophosphatidic acid acyltransferase, and FA-binding protein, associated with lipid transport synthesis and secretion, rose 2.6-5.1 folds higher. In contrast, apolipoproteins A1, C-III, E, and A-IV were 2.6- 4.3-fold less concentrated in milk than in MFG membranes isolated from colostrum. Despite higher fat contents in colostrum, there may be early development shifts in milk fat transport.

Effect of gastrointestinal microflora: Bacterial infections can alter immune-related proteins of the MFG membrane. Mastitis due to *Mycoplasma agalactiae* can initiate an immune response with induction of host defense, inflammation, and oxidative stress, and suppression of milk fat metabolism and secretion.[141] Similarly, neutrophils produce more extracellular traps and a stronger bactericidal response when exposed to *Staphylococcus aureus*; the related antimicrobial peptides are present in the MFG membrane fraction.[142]

The effects of dietary supplementation with microalgae to increase PUFAs on the bovine milk FA profile and the number and diameter of MFGs have also been investigated. Dietary supplementation with *Chlorella* reduced the number of 1–3 μm globules compared to other diets.[16] Dietary composition did not consistently change all MFG size fractions. Changes in capric (C10:0), lauric (C12:0), myristic (C14:0), and oleic acid (C18:1) concentrations were not predictable. The structure of human MFG and the specific positional distribution of FAs may explain differences in the gut microbiota between infants who are fed with HM vs formula. Human milk contains β-16:0 (palmitic acid esterified on the *sn-2* position) in contrast to vegetable-sourced palmitic acid, which is esterified in the *sn*-1 or–3 positions.[143] One study showed that supplementing formula with β-16:0 increased fecal abundance of Lactobacilli and Bifidobacteria in infants after 6 weeks of feeding compared to a control formula containing vegetable-sourced 16:0.[144] The mechanisms behind these observations need to be determined.

HM-fed infants normally show a predominance of *Firmicutes*. However, a combination of milk fat and MFG membrane fragments altered fecal microbial composition in piglets by increasing *Proteobacteria* and *Bacteroidetes* at the expense of *Firmicutes*.[145] This may partly be explained by an increased intestinal content of immune modulatory peptides and milk lipid-derived metabolites. Another study in germ-free mice showed that an infant formula high in medium-chain FAs emulsified with bovine MFG membranes enriched the bacterial families *Bacteriodaceae, Desulfovibrionaceae, Rikenellaceae*, and *Porphyromonadaceae*, whereas formulas made with LCFAs emulsified with soy lecithin increased the abundance of *Enterobacteriaceae, Erysipelotrichaceae, Coriobacteriaceae*, and *Enterococcaceae*.[20] These effects may correlate with the chain length and the degree of desaturation of the fatty acids. MFG lipids can influence the relative distribution of various protein digestion products that enter the colon by altering the rate of hydrolysis of proteins in the small intestine.[20] Further work is needed.

Proteins in the MFG membrane: Proteins constitute 25–70% of the total MFG membrane (w/w) with a high proportion of glycoproteins and enzymes.[131] In HM, MFG membrane proteins comprise about 1–4% w/w of the total milk proteins.[63] These proteins regulate cellular processes and defense mechanisms in the maternal-infant pair. Proteomic studies have mapped up to 400 proteins in HM MFG membranes.[146] However, the protein extraction methods still need work.

Proteins such as BTN; a member of the immunoglobulin superfamily), xanthine dehydrogenase/oxidase (XDH/XO), adipophilin (ADPF; perilipin-2, PLIN2), and stomatin are involved in MFG formation.[16] High MFG epidermal growth factor 8 (MFGE8, lactadherin), ADPF, and glycosylation-dependent cell adhesion molecule-1 (GLYCAM1): proteose peptone component 3 (PP3) can also promote the formation of large MFGs. PP3 (lactophorin), is a small phosphoglycoprotein that is expressed in lactating mammary tissue. In addition, α-lactalbumin, lysozyme, β-casein, clusterin, lactoferrin, immunoglobulins such as the IgA α-chain, tenascin, apolipoproteins such as type A-I, and FA synthase have been identified.

These proteins exert a wide spectrum of bioactive properties. For instance, ADPH controls the trafficking of lipids towards

the MFGs.[62] BTN is a member of the immunoglobulin superfamily, and it also connects the inner and the outer MFG membrane by binding XDH/XO and ADPH in a tripartite superstructure.[147] BTN1A1 is the main isoform in the human MFG membrane and regulates the lipid secretion in milk.[148] Mucin 1 (MUC1) is a glycoprotein that might bind pathogens as a decoy receptor.[149] XDH/XO is a redox enzyme that exerts its antimicrobial role by producing reactive oxygen and nitrogen species.[150] Lactadherin, also known as Per-Arnt-Sim (PAS) domain 6/7 or milk fat globule-epidermal growth factor-factor 8 (MFG-E8), is involved in the regulation of apoptosis and in innate immune responses.[151] FA-binding proteins (FABPs) typically transport long-chain FAs into the mammary epithelial cells.[152]

Many MFG membrane proteins regulate inflammatory reactions by altering cytokine expression, promoting apoptosis, and reducing oxidation.[153] Glycosylated compounds and the products of hydrolysis can protect against bacteria, viruses, and bacterial toxins.[154] Human MFG membrane contains human leukocyte antigen (HLA)-II, which is normally expressed on the surface of antigen-presenting cells.[20] This may promote antigen presentation to $CD4^+$ T-cells and promote immune responses/tolerance in the neonatal intestine.

Some MFG membrane proteins may be involved in T-cell maturation: (a) BTN in the MFG membranes suppresses the proliferation and activity of maternal and neonatal T-cells.[20] It also facilitates immune resolution following prolonged inflammation by clearing apoptotic cells; it binds phosphatidylserine on apoptotic cells via its C-terminal V/VIII-like domains, and its epidermal growth factor domain contains the arginine-glycine-aspartate (RGD) motif that interacts with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors to activate macrophages to clear the apoptotic débris;[20] (b) alkaline phosphatase in MFG membranes also has anti-inflammatory properties; it dephosphorylates pro-inflammatory molecules such as lipopolysaccharide (LPS), inhibiting TLR-mediated NF-κB signaling. Milk phospholipids and FAs, particularly LCFA, are strong stimulators of intestinal AP activity;[155] (c) osteopontin (OPN) in the MFG membrane activates the innate and adaptive immune systems of newborns. It works as an opsonin, binding directly to bacteria such as *Streptococcus agalactiae* and *S. aureus* to enhance clearance by macrophages. It also balances Th1 and Th2 immune responses.[20]

The mechanisms of MFG secretion from mammary epithelial cells are being investigated. A tripartite model proposes that interactions among the MFG membrane proteins, ADPF, XDH/XO, and BTN promote MFG secretion. These proteins presumably bind on the MFG surface and promote the adsorption of lipid droplets.[156] This complex leads to the deformation of the lipid droplets and the budding of more lipid droplets from the secretory system.

After release, MFGs may show some casein-balancing membrane loss. Caseins are known to be synthesized and secreted by the fusion of casein-containing vesicles with the apical plasma membrane and soluble *N*-ethylmaleimide–sensitive fusion attachment protein receptor (SNARE).[157] This model suggests even though BTN1, PLIN2, and XOR likely contribute to MFG budding, SNARE proteins connect the secretory vesicles together and with the apical plasma membrane to promote the exocytosis of the budding MFGs. The combined release of MFGs and casein micelles from the mammary epithelium may contribute to the adsorption of casein micelles on the MFG membrane. Further studies are needed to explore these possibilities.

Glycosylated mucin 1 and 15, ADPF, BTN, and XDH are more concentrated in MFG membrane in the smaller the MFG fraction. Increased glycosylation helps MFG membrane proteins resist digestion in the upper gastrointestinal tract and retain these biological effects in the colon.[16] Mucin and lactadherin promote mucosal immunity in the stomach and upper intestine and protect against bacteria and viruses.[158]

Many proteomic studies have listed several casein and whey proteins in the MFG membrane.[159] Casein digestion may produce several bioactive peptides, which have been linked to gastrointestinal and immunological function, neurodevelopment, and even the effectiveness of antibiotics and probiotics.[160] Lactoferrin, a whey protein, is detectable both bound to the MFGM and in the aqueous phase of milk.[11] Analyses using the Kyoto Encyclopedia of Genes and Genomes (KEGG) have listed proteins involved in cell signaling, membrane transport, immune responses, and protein metabolism. These studies differ in exact genes and variants, but these differences could have emanated from the use of different databases for categorizing proteins.[161] Human MFGM contains important immune response mediators such as lactoferrin, immunoglobulins (alpha and gamma chain C region), monocyte differentiation CD14, clusterin, toll-like receptor 2, mucin 4, cathelicidins, and dermcidin, which have been identified as important in immune response.[161] Clusterin and lactadherin also play a role in cell damage and apoptosis, and consequently, in gut epithelial homeostasis.[162] Hormone-sensitive lipase, peroxisomal bifunctional enzyme, peroxisomal multifunctional enzyme type 2, peroxisomal acyl-coenzyme A oxidase 3, carboxyl ester lipase (GD3, BSSL), and sphingomyelin phosphodiesterase promote lipid digestion.[16]

Ribonucleic acid (RNA) in the MFG membrane: The cytoplasmic crescent of the MFG membrane holds cellular and microRNAs (miRNAs).[163] This transcriptome varies with a circadian rhythm and at different stages of lactation, to alter metabolic and immune regulation. Key genes involved in lactose synthesis and insulin signaling are closely regulated.[164]

MFG Core

The core of MFGs is comprised of TAGs, which constitute nearly 98% (w/w) of the total milk lipids.[16] In addition to being an important source of energy, MFGs serve as a vehicle for fat-soluble vitamins and carotenoids. Some of the other important fat constituents in these globules include phospholipids, glycolipids, and sphingomyelin, which originate from the ER. Once matured, these globules are secreted across the apical membrane of the mammary epithelial cells.

Milk fat contains over 400 different FAs, of which 15 constitute 90% of the total FA pool.[63] Compared to the MFG membrane, most TAGs in the MFG core consist of 18:1 (*n*–9) oleic (20–35%), 16:0 palmitic (18–23%), and 18:2 (*n*–6) linoleic (LA; 8–18%) acids.[20] Medium chain FAs (MCFAs) comprise 12% of total FAs, and < 1% are short-chain FAs. TAGs contain nearly 85% of the milk LC-PUFAs such as 20:4 (*n*–6) ARA, 20:5 (*n*–3) eicosapentaenoic (EPA), and 22:6 (*n*–3) DHA. Even though the bioavailability of TAGs and phospholipid-bound LC-PUFAs might not differ, the metabolic disposition and incorporation into various organs differ. The 18:3 (*n*–3) α-linolenic acid (ALA) is less abundant, although there is a wide inter-individual variation. Small MFGs (1.6–3 μm) contain more medium-chain (C8-C12) but less long-chain saturated FAs such as stearic acid in their TAG core.

The location of FAs on the glycerol backbone is highly conserved within species.[165] Most saturated FAs are typically seen

on the *sn*-2 position of TAGs. Palmitic acid constitutes 50–60% of all FAs at the *sn*-2 position (defined as β-16:0) in HM. The *sn*-1 and *sn*-3 positions are occupied primarily by unsaturated FAs such as oleic acid.[3]

Milk lipid fraction also contains minerals and lipophilic vitamins.[166] These components are embedded in both the MFG core and the MFGM. The fat fraction acts as a "natural carrier" for these compounds, increasing their bioaccessibility and bioavailability. Understanding the micronutrient content of the fat fraction could lead to their technological exploitation for the design of value-added products, in concert with the MFGM proteome (that acts as a scaffold for the binding of minerals and vitamins to MFGs) and the bioactive polar lipids.

Digestion of MFGs

Milk fat digestion in infants is a sequential, balanced process. The lipase triad of lingual, gastric, and pancreatic origin may show some variability but is active.[167,168] In the stomach, digestive hydrolysis is catalyzed by specific enzymes. As the pH drops below 5.5, the MFG membrane structure becomes less stable and leads to coagulation of the fat globules.[14]

In HM, TAGs carry FAs located in selected areas.[3,169] Nearly 70% of the *sn*-2 positions are esterified with a saturated FA; palmitic acid (C16:0) is a leading ligand (20–25%). The *sn*-1,3 positions are occupied by unsaturated FAs. FA region distribution affects the kinetics of digestion because gastric lipase shows stereospecificity for the *sn*-3. Medium chain-FAs (C8–C12) are more frequently located in the *sn*-3 position. FAs esterified at the *sn*-2 position of TAG are important because nearly 70% of the FAs absorbed as *sn*-2 monoacylglycerols are absorbed across the enterocytes and conserved in the original position during re-esterification into TAGs for secretion into the plasma as chylomicrons. The higher efficacy of absorption of palmitate at *sn*-2 compared to those at the *sn*-1,3 positions is well known. The absorption of FAs decreases as the FA chain length or unsaturation degree increases in HM.

Gastric lipases hydrolyze 5–40% of the dietary lipids in MFGs by acting on the *sn*-3 position of the TAGs.[170] This releases short- to medium-chain FAs, which play important metabolic roles such as in the acylation of proteins. Human gastric lipases can digest lipid droplets covered by polar lipids such as in the MFGs. The effects of gastric lipases are reinforced by endogenous milk lipases such as the BSSL and lipoprotein lipase.[171] In HM, lipases have easier access to the surface and subsequently to the interior of small MFGs, which can affect lipid digestion rates.[16]

Smaller MFGs adsorb more lipases and are digested faster than the larger ones but the composition and structure of the surrounding interfacial layer also influences the rates of lipid digestion.[11] The interfacial composition of an emulsion critically influences the activity of pancreatic lipase. Emulsifiers such as proteins, phospholipids, and surfactants interact with lipase to release FAs and promote/inhibit the adsorption of lipase on the emulsion surface.

Infants could have been at risk of not being able to utilize dietary fats in MFGs because of immature digestive processes. However, there are many compensating processes at work. First, there could have been difficulties for the lipases to penetrate the outer lipid coverings of the MFGs and reach the fats in the core.[172] Here, multiple gastric enzymatic reactions help detach these layers.[173] Next, there are mechanisms to compensate for the developmental inefficiencies such as the smaller size and motility of digestive organs, differences in gastric pH, low activity of digestive enzymes, and dietary patterns. The anionic phospholipids in HM promote lipase adsorption on the globules.[174] Furthermore, a bile salt-activated lipase (BSSL) in HM adds to the activities of gastric lipase, pancreatic-lipase-related protein 2 (PLRP2), and the phospholipase A_2 to compensate for insufficient bile salts and pancreatic triacylglycerol lipases (PTLs).[175–178] BSSL can digest medium and long TAGs, diacylglycerols, and phospholipids. It can also hydrolyze cholesterol esters, phospholipids, and ceramide.[179] Dietary SM is hydrolyzed by alkaline sphingomyelinase into phosphocholine and ceramide.[180] Neutral ceramidase hydrolyzes ceramide to FAs and sphingosine.[181] The sphingosine released in this process is adsorbed, phosphorylated to sphingosine-1-phosphate (S1P), and then converted to palmitic acid via the S1P-lyase present in the gut mucosa.[182] All these alternative processes can achieve about 95% efficiency of digestive lipolysis. Nearly 40–70% of dietary lipids are hydrolyzed to release long-chain PUFAs (C20:4 *n*–6, 20:5 *n*–3 and C22:6 *n*–3).[183] The *sn*-1,3 hydrolysis of milk fats by the PTLs releases *sn*-2 mono palmitin and the externally-positioned oleic acid.[184]

In HM, fat globules covered with MFG membrane are readily hydrolyzed when exposed to colipase and phospholipase A2, but not with pancreatic lipase.[185] Phospholipids could be protective against the digestive action of pancreatic lipase; the effects vary with the stage of digestion, the exact part of the gastrointestinal tract, and the type of phospholipid.[186] Phospholipids stabilized MFGs during storage by suppressing the adsorption of whey proteins on the globule surface.[187] SM also plays an important role in this process. The digestion of SM is less efficient in the upper intestine; these are partially cleaved to ceramide and sphingosine by alkaline sphingomyelinases.[182] This limited capacity of SM digestion favors the formation of SM-cholesterol complexes in the proximal parts of the intestine and exposes the lower small intestine and colon to SM and its bioactive metabolites.

In mouse pups, the MFG structure promotes metabolic programming.[188] Pups were fed from postnatal days 16 and 42 with either a control infant formula with submicronic fat droplets covered with milk proteins or with a novel formula with MFG-like larger fat droplets. Subsequently, all mice were fed a high-fat diet. Despite similar food intake, mice fed with the newer formula with MFG-like fat droplets showed better metabolic responses, lower body weight, less adipose tissue, and lower plasma insulin. These differential responses may be partly explained by different postprandial trafficking of FAs during infancy, inducing different storage responses of the adipose tissues. However, a direct effect of MFG membrane in the concept formula is challenged by a recent study of human infants fed with a standard formula vs a formula enriched with MFG membrane fragments.[189] There were no differences in infant growth, weight gain, and body fat at 12 months and at 6.5 years.[189,190]

Diets containing MFG membranes likely promote cognitive development in infants. In one study, infants fed MFG membrane-enriched formula showed cognitive scores that were similar to those of breastfed infants but were significantly higher than those fed standard infant formula.[189] In another study, infants fed with an infant formula enriched with MFG membrane, LC-PUFAs, and synbiotics enriched infant formula-fed infants seem to show fewer behavioral problems up to 2.5 years compared to standard infant formula-fed infants.[191] A pilot randomized control trial showed that sphingomyelin-fortified milk improved the neurobehavioral development of very low birth weight infants during infancy.[138]

The intervention groups performed significantly better at 18 months in the Bayley Scales of Infant and Toddler Development (BSID)-II, the Fagan test scores (evaluate visual recognition memory, habituation, and discrimination; relate these to intellectual functioning later in life), latency of visual evoked potentials, and sustained attention test scores. Further work is needed to elucidate the mechanisms by which MFG membrane components improve cognitive function.

The digestion of MFGs may be altered by the ζ-potential, the electric potential resulting from shear at the surface of these globules.[192] MFGs with large positive or negative ζ-potentials are electrostatically more stable; those with small ζ-potentials tend to coagulate or flocculate.[40] Smaller ζ-potential MFGs in HM are digested more easily, but flocculation of these smaller MFGs can reduce the surface area and access to digestive enzymes and bile salts.[40] These properties may be altered by the mineral composition of the milk, and the glycoprotein and glycolipid composition of the MFG membrane. The ζ-potentials also change over time after birth; the values for colostrum, transitional, and mature MFG were -5.60 ± 0.12, -6.72 ± 0.16, and -7.25 ± 0.61 mV, respectively.[16,67,193] Mature human MFGs have smaller ζ-potentials than in other mammals.[16]

The term 'food matrix' refers to the specific organization of foods that critically influence the release and absorption of nutrients during digestion in the gastrointestinal tract.[194] FA release from liquid matrix is quicker than from semi-solids. Similarly, the MFG structure, particularly the MFG membrane matrix, influences the rate of lipid digestion. In infant formula, lipid digestion was increased when intact proteins were replaced with hydrolyzed proteins.[195] The digestibility of fat is affected by its physical state, whether it is solid fat or liquid oil.[196] Whey protein-stabilized emulsions containing high levels of solid fat (hydrogenated soybean oil, melting point >37°C) release fewer FFAs during intestinal digestion *in vitro*.[196]

Biological Effects

The addition of MFG membranes to the infant diet has shown beneficial health effects in several preliminary studies. In addition to infant formula (0.5 gm/L) accelerated neurodevelopment and promoted cognitive function in healthy full-term infants with a low incidence of pathogen-associated adverse effects.[197] Healthy 6-month-old infants fed infant formula fortified with milk polar lipids showed better hand-eye coordination and developmental quotients compared to controls fed standard infant formula.[127] However, there were no differences in cognitive development compared to healthy exclusively breastfed infants. In other studies, infants fed formula enriched with sphingomyelin (28–71 mg/mL) showed improved cerebral myelination.[198] SM metabolites such as cerebroside can cross the blood-brain barrier to promote myelination.[199] Noting the differences in term and preterm milk, SM could well play a role in promoting brain development in preterm or low-birth-weight infants.[200]

Milk fat globule membranes can promote the utilization of fats. Higher lipolysis and β-oxidation in early life prevents excessive weight gain later and lowers the risk of obesity.[11] In rats, supplementation with MFG membranes reduced adipogenesis and excessive weight gain by promoting brown fat formation in white adipose tissues.[13] Similar supplementation to high-fat diet-fed rats during pregnancy and lactation stimulated brown fat development in male offsprings.[201] MFG membrane supplementation during suckling reduced the risk of maternal high-fat diet-induced nonalcoholic fatty liver disease in mice, possibly due to less oxidative stress and restored mitochondrial function.[202] In another study, such intervention corrected the stunted skeletal growth of male offsprings at weaning and protected against abnormalities in bone microstructure and insulin resistance during adulthood.[203] Enhanced insulin-like growth factor-I activity may be a possible mechanism underlying these changes.

In formula-fed infants or rodent models, feedings supplemented with MFG membranes lead to notable changes in the fecal microbiome to simulate that of HM-fed infants.[204] In rodents, supplementation with MFG membranes increased the beta diversity of the cecal microbiome and improved spatial learning in the stress group.[205,206] The MFG membrane-mediated improved brain function may be related to the modulation of the gut microbiota via the gut-brain axis.[207] Similarly, in neonatal piglets, supplementation of infant formula with dairy MFG membranes and lipids influenced protein digestibility and microbiota composition.[208] In rats, MFG membrane supplementation promoted enterocyte proliferation and gut barrier function by inducing tight junction proteins.[11] In healthy term infants, MFG membrane components such as lactadherin (MFG-epidermal grow factor-8), sialic acid, and phospholipid promoted the growth of *Bifidobacterium* and suppressed the growth of *Veillonella*, *Escherichia* and *Shigella* spp.[209] These changes were relatively modest at 4 months, suggesting that age could be a confounder.

Administration of MFG membranes enhanced the intestinal barrier function in a rat model of short bowel syndrome.[210] Improved regulation of the NLRP6 inflammasome corrected gut dysbiosis.[210–212] Microbiota-modulated metabolites such as taurine, histamine, and spermine regulate the expression of the NLRP6 inflammasome, IL-18, and downstream antimicrobial peptide patterns in the intestine.[213] MFG membranes can also improve the viability of *Lactobacillus rhamnosus* GG from bile stress both *in vitro* and in murine models.[214] The promotion of probiotic survival by MFG membranes might provide a beneficial effect on gut health.[215]

Sphingomyelin, an important constituent of MFG membranes, affects lipid metabolism. When long-chain bases of SM were transported into cells via acyl-CoA synthetases, the uptake of long-chain-FAs was inhibited.[216] In the intestine, SM and its metabolites modulated inflammatory signaling.[137] Sphingosine-1-phosphate (S1P) improved endothelial cell survival and migration.[136,217] Furthermore, metabolites of dietary sphingolipids influenced gut microbiota with increased commensal bacteria.[218] The mechanisms of these observations need further study.

Milk fat globule membranes need to be observed for nutritional and safety consequences depending on the actual composition of these membrane fractions.[219] Lipid-rich MFG membrane supplementation ($n = 70$, age 14 days) for 14 weeks did not result in any major safety concerns such as weight gain, morbidity, and metabolic markers, whereas protein-rich MFG membrane enrichment ($n = 72$) was associated with a higher rate of atopic dermatitis than in controls ($n = 57$).[220] These results need to be interpreted cautiously because the evaluation of skin lesions was not standardized and was based on parental reports. Further studies are needed.

Undigested MFG membranes that reach the colon support the colonization of microbial communities.[204] MFG membrane phospholipids can enrich *Porphyromonadaceae*, which differs from the impact of soy lecithin on *Enterobacteriaceae* and *Enterococcaceae*.[20] In another study, rat pups fed a formula supplemented with bovine MFG membrane increased gut microbial species richness and evenness compared with those who were fed

a formula containing vegetable fat.[221] At the phylum level, the microbiota of rat pups fed MFG membrane resembled those reared on dam's milk with similar levels of *Firmicutes* and *Proteobacteria*. Control pups on regular formula showed more *Proteobacteria*.[222] In human infants, MFG membranes show intrinsic antimicrobial activity; a double-blind RCT showed that infants fed an experimental formula supplemented with bovine MFG membranes during 2–6 months of age experienced fewer acute otitis media infections than controls, fewer days with fever, a reduction in fever incidence, and improved behavioral outcomes.[153] Polar lipids such as sphingophospholipids and gangliosides in the MFG membrane also exhibit antimicrobial activities. In preterm newborns, infants fed a formula supplemented with bovine gangliosides showed smaller *E. coli* fecal counts enriched *Bifidobacterium* compared to a standard formula.[223]

XOR in the MFG membrane generates reactive oxygen and nitrogen species and can inhibit the growth of *E. coli* and *Salmonella enteritides*.[20] α-Lactalbumin, a minor protein in the MFG membrane, is digested by pepsin, trypsin, and chymotrypsin in the intestine to generate bactericidal peptides that activate leukocytes.[224] Lysozyme, another MFG membrane protein, can protect against both Gram-positive and -negative bacteria due to the presence of 1,4-β-N-acetylmuraminidase which can degrade bacterial cell walls.[225] These effects of lysozyme are supported by lactoferrin, which carries iron and interacts with the lipid A moiety of LPS to damage the bacterial membrane.[20]

Milk fat globule membrane glycobiome is also potentially important in anti-bacterial defenses.[20] The MFG membrane contains glycoconjugates (glycolipids and glycoproteins) harboring both *N*-linked and *O*-lined glycan moieties.[20] The glycosylation patterns of these glycoconjugates in milk determine resistance to certain bacteria from binding specific mucosal receptors. The glycoproteins (MUC1, lactadherin) and gangliosides of the MFGM have the ability to interfere with such attachment. Mucin can also inhibit *Salmonella enterica serovar Typhimurium SL1344*, S-fimbriated *E. coli*, and rotavirus.[226,227] Further work is needed.

In infants, MFG membrane can bind and carry ingested probiotics such as Lactobacilli and *Bifidobacteria* to the colon.[228] HM bacteria can also subserve this function during the early stages of gut development Select OTUs assigned to *Bifidobacterium*, such as *B. breve, B. bifidum*, and *B. longum* that were identified in mother-infant pairs could preferentially associate with the MFG membrane utilizing glycan adhesion factors that enabled binding with mucin.[20] Increasing information indicates that the lactic acid bacteria (LAB), which include the phylum Firmicutes, class Bacilli, and order Lactobacillales, are comprised of 6 families: *Aerococcaceae*, *Carnobacteriaceae*, *Enterococcaceae*, *Lactobacillaceae*, *Leuconostocaceae*, and *Streptococcaceae*.[229] MFG membrane glycoproteins can survive gastric digestion and can show prebiotic effects to support the growth of colonic bacteria such as *Ruminococcus* and *Bifidobacteria* genera, most likely due to sialic acid residues.[230] The *Ruminococci* are important mutualist gut bacteria that serve to degrade and convert complex polysaccharides into a variety of nutrients for their hosts.[231]

Conclusion

The size and composition of MFGs may be influenced by maternal ethnicity, genetic factors, physiological state, diet, stage of lactation, and metabolic state.[17] In addition to these identified sources of diversity, there are still unexplained individual variations. Post-secretion modifications such as membrane vesiculation or MFG fusion may also affect MFG size and structure.[232] More data are needed to understand the *in vivo* digestibility of the MFG size fractions, and to clarify the release and activity of the MFG membrane.[233] Understanding the composition and dynamics of MFGs could help not only for personalized nutrition for chronic diseases, but also as an important channel for enteral delivery of medications as needed and at desired rates of release.[233] Wide use of nutraceuticals will become a closer reality.[233]

Authors Contribution

AM wrote the manuscript; HM, MMR, NB, AFB, JB added key components. All the authors critically reviewed and approved the manuscript prior to submission.

Orcid

Akhil Maheshwari https://orcid.org/0000-0003-3613-4054

References

1. Ballard O, Morrow AL. Human milk composition: Nutrients and bioactive factors. Pediatr Clin North Am 2013;60(1):49–74. DOI: 10.1016/j.pcl.2012.10.002.
2. Samuel TM, Zhou Q, Giuffrida F, et al. Nutritional and non-nutritional composition of human milk is modulated by maternal, infant, and methodological factors. Front Nutr 2020;7:576133. DOI: 10.3389/fnut.2020.576133.
3. Maheshwari A. Fats in human milk: 2022 updates on chemical composition. Newborn (Clarksville) 2022;1(4):384–396. DOI: 10.5005/jp-journals-11002-0050.
4. Valverde R, Dinerstein NA, Vain N. Mother's own milk and donor milk. World Rev Nutr Diet 2021;122:212–224. DOI: 10.1159/000514733.
5. Butte NF. Fat intake of children in relation to energy requirements. Am J Clin Nutr 2000;72(5 Suppl):1246S–1252S. DOI: 10.1093/ajcn/72.5.1246s.
6. Young BE, Krebs NF. Complementary feeding: Critical considerations to optimize growth, nutrition, and feeding behavior. Curr Pediatr Rep 2013;1(4):247–256. DOI: 10.1007/s40124-013-0030-8.
7. WHO U, USAID A, AED U. Indicators for assessing infant and young child feeding practices. Geneva: World Health Organization. 2008.
8. Argov N, Lemay DG, German JB. Milk fat globule structure & function; nanosciece comes to milk production. Trends Food Sci Technol 2008;19(12):10. DOI: 10.1016/j.tifs.2008.07.006.
9. Argov N, Wachsmann-Hogiu S, Freeman SL, et al. Size-dependent lipid content in human milk fat globules. J Agric Food Chem 2008;56(16):7446–7450. DOI: 10.1021/jf801026a.
10. Cavaletto M, Givonetti A, Cattaneo C. The immunological role of milk fat globule membrane. Nutrients 2022;14(21):4574. DOI: 10.3390/nu14214574.
11. Chai C, Oh S, Imm JY. Roles of milk fat globule membrane on fat digestion and infant nutrition. Food Sci Anim Resour 2022;42(3):351–371. DOI: 10.5851/kosfa.2022.e11.
12. Huang QX, Yang J, Hu M, et al. Milk fat globule membrane proteins are involved in controlling the size of milk fat globules during conjugated linoleic acid-induced milk fat depression. J Dairy Sci 2022;105(11):9179–9190. DOI: 10.3168/jds.2022-22131.
13. Li T, Gao J, Du M, et al. Milk fat globule membrane attenuates high-fat diet-induced obesity by inhibiting adipogenesis and increasing uncoupling protein 1 expression in white adipose tissue of mice. Nutrients 2018;10(3):331. DOI: 10.3390/nu10030331.
14. Lopez C. Milk fat globules enveloped by their biological membrane: Unique colloidal assemblies with a specific composition and structure. Curr Opin Colloid Interface Sci 2011;16(5)391–404. DOI: 10.1016/j.cocis.2011.05.007.

15. Sun Y, Roos YH, Miao S. Changes in milk fat globules and membrane proteins prepared from pH-adjusted bovine raw milk. Foods 2022;11(24):4107. DOI: 10.3390/foods11244107.
16. Thum C, Roy NC, Everett DW, et al. Variation in milk fat globule size and composition: A source of bioactives for human health. Crit Rev Food Sci Nutr 2023;63(1):87–113. DOI: 10.1080/10408398.2021.1944049.
17. Thum C, Wall C, Day L, et al. Changes in human milk fat globule composition throughout lactation: A review. Front Nutr 2022; 9:835856. DOI: 10.3389/fnut.2022.835856.
18. Walter L, Narayana VK, Fry R, et al. Milk fat globule size development in the mammary epithelial cell: A potential role for ether phosphatidylethanolamine. Sci Rep 2020;10(1):12299. DOI: 10.1038/s41598-020-69036-5.
19. McManaman JL. Lipid transport in the lactating mammary gland. J Mammary Gland Biol Neoplasia 2014;19(1):35–42. DOI: 10.1007/s10911-014-9318-8.
20. Lee H, Padhi E, Hasegawa Y, et al. Compositional dynamics of the milk fat globule and its role in infant development. Front Pediatr 2018;6:313. DOI: 10.3389/fped.2018.00313.
21. Newburg DS, Peterson JA, Ruiz-Palacios GM, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. Lancet 1998;351(9110):1160–1164. DOI: 10.1016/s0140-6736(97)10322-1.
22. Hahn-Holbrook J, Fish A, Glynn LM. Human milk omega-3 fatty acid composition is associated with infant temperament. Nutrients 2019;11(12):2964. DOI: 10.3390/nu11122964.
23. Ding D, He X, Agarry IE, et al. Profile of human milk phospholipids at different lactation stages with UPLC/Q-TOF-MS: Characterization, distribution, and differences. J Agric Food Chem 2023;71(16):6326–6337. DOI: 10.1021/acs.jafc.2c07512.
24. Seki D, Errerd T, Hall LJ. The role of human milk fats in shaping neonatal development and the early life gut microbiota. Microbiome Res Rep 2023;2(2):8. DOI: 10.20517/mrr.2023.09.
25. Boquien CY. Human milk: An ideal food for nutrition of preterm newborn. Front Pediatr 2018;6:295. DOI: 10.3389/fped.2018.00295.
26. Mead MN. Contaminants in human milk: Weighing the risks against the benefits of breastfeeding. Environ Health Perspect 2008;116(10):A427–A434. PMID: 18941560.
27. Jukkola A, Partanen R, Rojas OJ, et al. Separation of milk fat globules via microfiltration: Effect of diafiltration media and opportunities for stream valorization. J Dairy Sci 2016;99(11):8644–8654. DOI: 10.3168/jds.2016-11422.
28. Wiking L, Gregersen SB, Hansen SF, et al. Heat-induced changes in milk fat and milk fat globules and its derived effects on acid dairy gelation–A review. Int Dairy J 2022;127:105213. DOI: 10.1016/j.idairyj.2021.105213.
29. Raza GS, Herzig KH, Leppaluoto J. Invited review: Milk fat globule membrane-A possible panacea for neurodevelopment, infections, cardiometabolic diseases, and frailty. J Dairy Sci 2021;104(7): 7345–7363. DOI: 10.3168/jds.2020-19649.
30. Steffen F, Hansen BP, Rasmussen JT, et al. Placing pasteurisation before or after microfiltration impacts the protein composition of milk fat globule membrane material. Int Dairy J 2018;81:35–41. DOI: 10.1016/j.idairyj.2017.12.015.
31. Alshehab M, Reis MG, Day L, et al. Milk fat globules, a novel carrier for delivery of exogenous cholecalciferol. Food Res Int 2019;126:108579. DOI: 10.1016/j.foodres.2019.108579.
32. Acevedo-Fani A, Dave A, Singh H. Nature-assembled structures for delivery of bioactive compounds and their potential in functional foods. Front Chem 2020;8:564021. DOI: 10.3389/fchem.2020.564021.
33. Rahman MM, Khatun S, Kabir N, et al. Establishment of the first religiously-compliant human milk bank in bangladesh. Newborn (Clarksville) 2022;1(4):376–383. DOI: 10.5005/jp-journals-11002-0047.
34. Raz C, Paramonov MM, Shemesh M, et al. The milk fat globule size governs a physiological switch for biofilm formation by Bacillus subtilis. Front Nutr 2022;9:844587. DOI: 10.3389/fnut.2022.844587.
35. Masedunskas A, Chen Y, Stussman R, et al. Kinetics of milk lipid droplet transport, growth, and secretion revealed by intravital imaging: Lipid droplet release is intermittently stimulated by oxytocin. Mol Biol Cell 2017;28(7):935–946. DOI: 10.1091/mbc.E16-11-0776.
36. Di Marzo L, Cree P, Barbano DM. Prediction of fat globule particle size in homogenized milk using Fourier transform mid-infrared spectra. J Dairy Sci 2016;99(11):8549–8560. DOI: 10.3168/jds.2016-11284.
37. Kowalczuk PB, Drzymala J. Physical meaning of the Sauter mean diameter of spherical particulate matter. Particulate Science and Technology 2016;34(6):645–647. DOI: 10.1080/02726351.2015.1099582.
38. Lyu F, Thomas M, Hendriks WH, et al. Size reduction in feed technology and methods for determining, expressing and predicting particle size: A review. Animal Feed Science and Technology 2020;261:114347. DOI: https://DOI.org/10.1016/j.anifeedsci.2019.114347.
39. Pinto G, Baptista A, Silva F, et al. Study on the influence of the ball material on abrasive particles' dynamics in ball-cratering thin coatings wear tests. Materials (Basel) 2021;14(3):668. DOI: 10.3390/ma14030668.
40. Michalski MC, Briard V, Michel F, et al. Size distribution of fat globules in human colostrum, breast milk, and infant formula. J Dairy Sci 2005;88(6):1927–1940. DOI: 10.3168/jds.S0022-0302(05)72868-X.
41. Wei W, Li D, Jiang C, et al. Phospholipid composition and fat globule structure II: Comparison of mammalian milk from five different species. Food Chem 2022;388:132939. DOI: 10.1016/j.foodchem.2022.132939.
42. Anto L, Warykas SW, Torres-Gonzalez M, et al. Milk polar lipids: Underappreciated lipids with emerging health benefits. Nutrients 2020;12(4):1001. DOI: 10.3390/nu12041001.
43. Aumeistere L, Ciprovica I, Zavadska D, et al. Impact of maternal diet on human milk composition among lactating women in latvia. Medicina (Kaunas) 2019;55(5):173. DOI: 10.3390/medicina55050173.
44. Verardo V, Gomez-Caravaca AM, Arraez-Roman D, et al. Recent advances in phospholipids from colostrum, milk and dairy by-products. Int J Mol Sci 2017;18(1):173. DOI: 10.3390/ijms18010173.
45. Tan S, Chen C, Zhao A, et al. The dynamic changes of gangliosides in breast milk and the intake of gangliosides in maternal and infant diet in three cities of China. Int J Clin Exp Pathol 2020;13(11):2870–2888. PMID: 33284868.
46. Kolter T. Ganglioside biochemistry. ISRN Biochem 2012;2012:506160. DOI: 10.5402/2012/506160.
47. Mansson HL. Fatty acids in bovine milk fat. Food Nutr Res 2008;52(1). DOI: 10.3402/fnr.v52i0.1821.
48. Contarini G, Povolo M. Phospholipids in milk fat: Composition, biological and technological significance, and analytical strategies. Int J Mol Sci 2013;14(2):2808–2831. DOI: 10.3390/ijms14022808.
49. He X, McClorry S, Hernell O, et al. Digestion of human milk fat in healthy infants. Nutr Res 2020;83:15–29. DOI: 10.1016/j.nutres.2020.08.002.
50. Linn JG. Factors affecting the composition of milk from dairy cows. In: Products NRCUCoTOtItNAoA (Eds). Designing Foods: Animal Product Options in the Marketplace. Washington (DC): National Academies Press (US); 1988.
51. Sanchez-Hernandez S, Esteban-Munoz A, Gimenez-Martinez R, et al. A comparison of changes in the fatty acid profile of human milk of spanish lactating women during the first month of lactation using gas chromatography-mass spectrometry. A comparison with infant formulas. Nutrients 2019;11(12):3055. DOI: 10.3390/nu11123055.
52. Yang F, Chen G. The nutritional functions of dietary sphingomyelin and its applications in food. Front Nutr 2022;9:1002574. DOI: 10.3389/fnut.2022.1002574.
53. Sinanoglou VJ, Cavouras D, Boutsikou T, et al. Factors affecting human colostrum fatty acid profile: A case study. PLoS One 2017;12(4):e0175817. DOI: 10.1371/journal.pone.0175817.
54. Jia W, Zhang R, Zhu Z, et al. A high-throughput comparative proteomics of milk fat globule membrane reveals breed and lactation stages specific variation in protein abundance and functional differences between milk of saanen dairy goat and holstein bovine. Front Nutr 2021;8:680683. DOI: 10.3389/fnut.2021.680683.
55. Schultz-Pernice I, Engelbrecht LK, Petricca S, et al. Morphological analysis of human milk membrane enclosed structures reveals diverse

cells and cell-like milk fat globules. J Mammary Gland Biol Neoplasia 2020;25(4):397–408. DOI: 10.1007/s10911-020-09472-1.
56. Mulder H, Walstra P. The milk fat globule: Commonwealth Agricultural Bureaux Farnham Royal; 1974. pp. 54–97.
57. Andrews AT, Anderson M, Goodenough PW. A study of the heat stabilities of a number of indigenous milk enzymes. Journal of Dairy Research 1987;54(2):237–246. DOI: https://DOI.org/10.1017/S0022029900025371.
58. Hansen SF, Larsen LB, Wiking L. Thermal effects on IgM-milk fat globule-mediated agglutination. Journal of Dairy Research 2019;86(1):108–113. DOI: 10.1017/S0022029918000778.
59. Burge K, Vieira F, Eckert J, et al. Lipid composition, digestion, and absorption differences among neonatal feeding strategies: Potential implications for intestinal inflammation in preterm infants. Nutrients 2021;13(2):550. DOI: 10.3390/nu13020550.
60. Rogalska E, Ransac S, Verger R. Stereoselectivity of lipases. II. Stereoselective hydrolysis of triglycerides by gastric and pancreatic lipases. J Biol Chem 1990;265(33):20271–20276. PMID: 2243091.
61. Gimenez MS, Oliveros LB, Gomez NN. Nutritional deficiencies and phospholipid metabolism. Int J Mol Sci 2011;12(4):2408–2433. DOI: 10.3390/ijms12042408.
62. Manoni M, Di Lorenzo C, Ottoboni M, et al. Comparative proteomics of milk fat globule membrane (MFGM) proteome across species and lactation stages and the potentials of MFGM fractions in infant formula preparation. Foods 2020;9(9):1251. DOI: 10.3390/foods9091251.
63. Wang C, Qiao X, Gao Z, et al. Advancement on milk fat globule membrane: Separation, identification, and functional properties. Front Nutr 2021;8:807284. DOI: 10.3389/fnut.2021.807284.
64. Hansen SF, Hogan SA, Tobin J, et al. Microfiltration of raw milk for production of high-purity milk fat globule membrane material. J Food Eng 2020;276:109887. DOI: 10.1016/j.jfoodeng.2019.109887.
65. Hu Y, Thaler J, Nieuwland R. Extracellular vesicles in human milk. Pharmaceuticals (Basel) 2021;14(10):1050. DOI: 10.3390/ph14101050.
66. Argov-Argaman N, Smilowitz JT, Bricarello DA, et al. Lactosomes: Structural and compositional classification of unique nanometer-sized protein lipid particles of human milk. J Agric Food Chem 2010;58(21):11234–11242. DOI: 10.1021/jf102495s.
67. Mizuno K, Nishida Y, Taki M, et al. Is increased fat content of hindmilk due to the size or the number of milk fat globules? Int Breastfeed J 2009;4:7. DOI: 10.1186/1746-4358-4-7.
68. Argov-Argaman N, Raz C, Roth Z. Progesterone regulation of milk fat globule size is VLDL dependent. Front Endocrinol (Lausanne) 2020;11:596. DOI: 10.3389/fendo.2020.00596.
69. Churakov M, Karlsson J, Edvardsson Rasmussen A, et al. Milk fatty acids as indicators of negative energy balance of dairy cows in early lactation. Animal 2021;15(7):100253. DOI: 10.1016/j.animal.2021.100253.
70. Capuco AV, Akers RM. The origin and evolution of lactation. J Biol 2009;8(4):37. DOI: 10.1186/jbiol139.
71. Oftedal OT. The evolution of milk secretion and its ancient origins. Animal 2012;6(3):355–368. DOI: 10.1017/S1751731111001935.
72. Oftedal OT. The mammary gland and its origin during synapsid evolution. J Mammary Gland Biol Neoplasia 2002;7(3):225–252. DOI: 10.1023/a:1022896515287.
73. Oftedal OT. The origin of lactation as a water source for parchment-shelled eggs. J Mammary Gland Biol Neoplasia 2002;7(3):253–266. DOI: 10.1023/a:1022848632125.
74. Folk GE Jr, Semken HA Jr. The evolution of sweat glands. Int J Biometeorol 1991;35(3):180–186. DOI: 10.1007/BF01049065.
75. Lefevre CM, Sharp JA, Nicholas KR. Evolution of lactation: Ancient origin and extreme adaptations of the lactation system. Annu Rev Genomics Hum Genet 2010;11:219–238. DOI: 10.1146/annurev-genom-082509-141806.
76. Kemp TS. The origin and early radiation of the therapsid mammal-like reptiles: A palaeobiological hypothesis. J Evol Biol 2006;19(4):1231–1247. DOI: 10.1111/j.1420-9101.2005.01076.x.
77. Pavlova T, Spacil Z, Vidova V, et al. Adipophilin and perilipin 3 positively correlate with total lipid content in human breast milk. Sci Rep 2020;10(1):360. DOI: 10.1038/s41598-019-57241-w.
78. Chong BM, Reigan P, Mayle-Combs KD, et al. Determinants of adipophilin function in milk lipid formation and secretion. Trends Endocrinol Metab 2011;22(6):211–217. DOI: 10.1016/j.tem.2011.04.003.
79. Haarsma L, Nelesen S, VanAndel E, et al. Simulating evolution of protein complexes through gene duplication and co-option. J Theor Biol 2016;399:22–32. DOI: 10.1016/j.jtbi.2016.03.028.
80. Wells JN, Bergendahl LT, Marsh JA. Co-translational assembly of protein complexes. Biochem Soc Trans 2015;43(6):1221–1226. DOI: 10.1042/BST20150159.
81. Verma HC. Concepts of physics part I. 4th edition. New Delhi, India: S. Chand & Company Ltd; 2008. pp. 284–286.
82. Lewis M. Food dimensions; size, shape and numbers. Sawston, United Kingdom: Woodhead Publishing; 2023. pp. 12–86.
83. Koroleva M, Tokarev A, Yurtov E. Modeling droplet aggregation and percolation clustering in emulsions. Arab J Chem 2019;12(8):4458–4465. DOI: 10.1016/j.arabjc.2016.07.001.
84. Leong K, Wang F. A molecular dynamics investigation of the surface tension of water nanodroplets and a new technique for local pressure determination through density correlation J Chem Phys 2018;148(14):144503. DOI: 10.1063/1.5004985.
85. Colombo M, Wright C. First principles in the life sciences: The free-energy principle, organicism, and mechanism. Synthese 2021;198(14):3463–3488. DOI: 10.1007/s11229-018-01932-w.
86. Panchal BR, Truong T, Prakash S, et al. Effect of fat globule size on the churnability of dairy cream. Food Res Int 2017;99(Pt 1):229–338. DOI: 10.1016/j.foodres.2017.05.027.
87. Davies-Jones R. An expression for effective buoyancy in surroundings with horizontal density gradients. J Atmos Sci 2003;60(23):2922–2955. DOI: 10.1175/1520-0469(2003)060<2922:AEFEBI>2.0.CO;2.
88. Bannon P. Theoretical foundations for models of moist convection. J Atmos Sci 2002;59(12):1967–1982. DOI: https://DOI.org/10.1175/1520-0469(2002)059<1967:TFFMOM>2.0.CO;2.
89. Das P. A non-archimedean approach to the equations of convection dynamics. J Atmos Sci 1979;36(11):2183–2190. DOI: 10.1175/1520-0469(1979)036<2183:ANAATT>2.0.CO;2.
90. Wang L, Chen W, Guo H. Response of membrane tension to gravity in an approximate cell model. Theor Biol Med Model 2019;16(1):19. DOI: 10.1186/s12976-019-0116-2.
91. Dey S, Zeeshan Ali S, Padhi E. Terminal fall velocity: The legacy of Stokes from the perspective of fluvial hydraulics. Proc Math Phys Eng Sci 2019;475(2228):20190277. DOI: 10.1098/rspa.2019.0277.
92. Cartwright JHE. Stokes' law, viscometry, and the Stokes falling sphere clock. Philos Trans A Math Phys Eng Sci 2020;378(2179):20200214. DOI: 10.1098/rsta.2020.0214.
93. Gomez-Solano JR, July C, Mehl J, et al. Non-equilibrium work distribution for interacting colloidal particles under friction. New J Phys 2015;17:045026. DOI: 10.1088/1367-2630/17/4/045026.
94. Smoczynski M. Role of phospholipid flux during milk secretion in the mammary gland. J Mammary Gland Biol Neoplasia 2017;22(2):117–129. DOI: 10.1007/s10911-017-9376-9.
95. D'Incecco P, Ong L, Pellegrino L, et al. Effect of temperature on the microstructure of fat globules and the immunoglobulin-mediated interactions between fat and bacteria in natural raw milk creaming. J Dairy Sci 2018;101(4):2984–2997. DOI: 10.3168/jds.2017-13580.
96. Wu C, Qian T, Sheng P. Droplet spreading driven by van der Waals force: a molecular dynamics study. J Phys Condens Matter 2010;22(32):325101. DOI: 10.1088/0953-8984/22/32/325101.
97. Lahiri R, Arvind, Sain A. Brownian motion in a classical ideal gas: A microscopic approach to Langevin's equation. Pramana - J Phys 2004;62:1015–1028. DOI: 10.1007/BF02705249.
98. Rings D, Schachoff R, Selmke M, et al. Hot brownian motion. Phys Rev Lett 2010;105(9):090604. DOI: 10.1103/PhysRevLett.105.090604.
99. Mokshin AV, Yulmetyev RM, Hänggi P. Diffusion processes and memory effects. New J Phys 2005;7:9. DOI: 10.1088/1367-2630/7/1/009.

100. Pitre L, Plimmer MD, Sparasci F, et al. Determinations of the Boltzmann constant. Comptes Rendus Physique 2019;20(1–2):129–139. DOI: 10.1016/j.crhy.2018.11.007.
101. de Oliveira MJ. Boltzmann stochastic thermodynamics. Phys Rev E 2019;99(5–1):052138. DOI: 10.1103/PhysRevE.99.052138.
102. Sinha KP, Das S, Karyappa RB, et al. Electrohydrodynamics of vesicles and capsules. Langmuir 2020;36(18):4863–4886. DOI: 10.1021/acs.langmuir.9b03971.
103. Wei T, Huang Y, Weng C, et al. Lipid rafts may affect the coalescence of milk fat globules through phase transition after thermal treatment. Food Chem 2023;399:133867. DOI: 10.1016/j.foodchem.2022.133867.
104. Pan J, Chen M, Li N, et al. Bioactive functions of lipids in the milk fat globule membrane: A comprehensive review. Foods 2023;12(20):3755. DOI: 10.3390/foods12203755.
105. Lu J, Argov-Argaman N, Anggrek J, et al. The protein and lipid composition of the membrane of milk fat globules depends on their size. J Dairy Sci 2016;99(6):4726–4738. DOI: 10.3168/jds.2015-10375.
106. Wooding FB, Mather IH. Ultrastructural and immunocytochemical evidence for the reorganisation of the milk fat globule membrane after secretion. Cell Tissue Res 2017;367(2):283–295. DOI: 10.1007/s00441-016-2505-8.
107. Yasuda T, Al Sazzad MA, Jantti NZ, et al. The influence of hydrogen bonding on sphingomyelin/colipid interactions in bilayer membranes. Biophys J 2016;110(2):431–440. DOI: 10.1016/j.bpj.2015.11.3515.
108. Smoczyński M, Staniewski B, Kiełczewska K. Composition and structure of the bovine milk fat globule membrane—Some nutritional and technological implications. Food Reviews International 2012;28(2):188–202. DOI: 10.1080/87559129.2011.595024.
109. McManaman JL. Formation of milk lipids: A molecular perspective. Clin Lipidol 2009;4(3):391–401. DOI: 10.2217/clp.09.15.
110. Spitsberg VL, Matitashvili E, Gorewit RC. Association and coexpression of fatty-acid-binding protein and glycoprotein CD36 in the bovine mammary gland. Eur J Biochem 1995;230(3):872–878. DOI: 10.1111/j.1432-1033.1995.tb20630.x.
111. Yang Y, Spitzer E, Kenney N, et al. Members of the fatty acid binding protein family are differentiation factors for the mammary gland. J Cell Biol 1994;127(4):1097–1109. DOI: 10.1083/jcb.127.4.1097.
112. Reinhardt TA, Lippolis JD. Developmental changes in the milk fat globule membrane proteome during the transition from colostrum to milk. J Dairy Sci 2008;91(6):2307–2318. DOI: 10.3168/jds.2007-0952.
113. Yang MT, Lan QY, Liang X, et al. Lactational changes of phospholipids content and composition in chinese breast milk. Nutrients 2022;14(8):1539. DOI: 10.3390/nu14081539.
114. Ma L, Fong BY, MacGibbon AKH, et al. Qualitative and quantitative study of glycosphingolipids in human milk and bovine milk using high performance liquid chromatography-data-dependent acquisition-mass spectrometry. Molecules 2020;25(17):4024. DOI: 10.3390/molecules25174024.
115. Nilsson A, Duan RD. Absorption and lipoprotein transport of sphingomyelin. J Lipid Res 2006;47(1):154–171. DOI: 10.1194/jlr.M500357-JLR200.
116. Nieto-Ruiz A, Garcia-Santos JA, Verdejo-Roman J, et al. Infant formula supplemented with milk fat globule membrane, long-chain polyunsaturated fatty acids, and synbiotics is associated with neurocognitive function and brain structure of healthy children aged 6 years: The COGNIS study. Front Nutr 2022;9:820224. DOI: 10.3389/fnut.2022.820224.
117. Jiang B, Xia Y, Zhou L, et al. Safety and tolerance assessment of milk fat globule membrane-enriched infant formulas in healthy term Chinese infants: A randomised multicenter controlled trial. BMC Pediatr 2022;22(1):465. DOI: 10.1186/s12887-022-03507-8.
118. Ambrozej D, Dumycz K, Dziechciarz P, et al. Milk fat globule membrane supplementation in children: Systematic review with meta-analysis. Nutrients 2021;13(3):714. DOI: 10.3390/nu13030714.
119. Khor GL, Tan SS, Stoutjesdijk E, et al. Temporal changes in breast milk fatty acids contents: A case study of malay breastfeeding women. Nutrients 2020;13(1):101. DOI: 10.3390/nu13010101.
120. Delplanque B, Gibson R, Koletzko B, et al. Lipid quality in infant nutrition: Current knowledge and future opportunities. J Pediatr Gastroenterol Nutr 2015;61(1):8–17. DOI: 10.1097/MPG.0000000000000818.
121. Hadley KB, Ryan AS, Forsyth S, et al. The essentiality of arachidonic acid in infant development. Nutrients 2016;8(4):216. DOI: 10.3390/nu8040216.
122. Li J, Pora BLR, Dong K, et al. Health benefits of docosahexaenoic acid and its bioavailability: A review. Food Sci Nutr 2021;9(9):5229–5243. DOI: 10.1002/fsn3.2299.
123. Rueda R. The role of dietary gangliosides on immunity and the prevention of infection. Br J Nutr 2007;98 Suppl 1:S68–S73. DOI: 10.1017/S0007114507832946.
124. Schnaar RL, Gerardy-Schahn R, Hildebrandt H. Sialic acids in the brain: Gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. Physiol Rev 2014;94(2):461–518. DOI: 10.1152/physrev.00033.2013.
125. Bronnum H, Seested T, Hellgren LI, et al. Milk-derived GM(3) and GD(3) differentially inhibit dendritic cell maturation and effector functionalities. Scand J Immunol 2005;61(6):551–557. DOI: 10.1111/j.1365-3083.2005.01566.x.
126. Palmano K, Rowan A, Guillermo R, et al. The role of gangliosides in neurodevelopment. Nutrients 2015;7(5):3891–3913. DOI: 10.3390/nu7053891.
127. Gurnida DA, Rowan AM, Idjradinata P, et al. Association of complex lipids containing gangliosides with cognitive development of 6-month-old infants. Early Hum Dev 2012;88(8):595–601. DOI: 10.1016/j.earlhumdev.2012.01.003.
128. Venkat M, Chia LW, Lambers TT. Milk polar lipids composition and functionality: A systematic review. Crit Rev Food Sci Nutr 2024;64(1):31–75. DOI: 10.1080/10408398.2022.2104211.
129. Okuda T. Dietary control of ganglioside expression in mammalian tissues. Int J Mol Sci 2019;21(1):177. DOI: 10.3390/ijms21010177.
130. Park EJ, Suh M, Thomson AB, et al. Dietary gangliosides increase the content and molecular percentage of ether phospholipids containing 20:4n-6 and 22:6n-3 in weanling rat intestine. J Nutr Biochem 2006;17(5):337–344. DOI: 10.1016/j.jnutbio.2005.08.005.
131. Dewettinck K, Rombaut R, Thienpont N, et al. Nutritional and technological aspects of milk fat globule membrane material. Int Dairy J 2008;18(5):436–457. DOI: 10.1016/j.idairyj.2007.10.014.
132. Gomez-Larrauri A, Presa N, Dominguez-Herrera A, et al. Role of bioactive sphingolipids in physiology and pathology. Essays Biochem 2020;64(3):579–589. DOI: 10.1042/EBC20190091.
133. Lee M, Lee SY, Bae YS. Functional roles of sphingolipids in immunity and their implication in disease. Exp Mol Med 2023;55(6):1110–1130. DOI: 10.1038/s12276-023-01018-9.
134. Obinata H, Hla T. Sphingosine 1-phosphate and inflammation. Int Immunol 2019;31(9):617–625. DOI: 10.1093/intimm/dxz037.
135. Et-Thakafy O, Guyomarc'h F, Lopez C. Lipid domains in the milk fat globule membrane: Dynamics investigated in situ in milk in relation to temperature and time. Food Chem 2017;220:352–361. DOI: 10.1016/j.foodchem.2016.10.017.
136. Dei Cas M, Paroni R, Signorelli P, et al. Human breast milk as source of sphingolipids for newborns: Comparison with infant formulas and commercial cow's milk. J Transl Med 2020;18(1):481. DOI: 10.1186/s12967-020-02641-0.
137. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. Nature 2014;510(7503):58–67. DOI: 10.1038/nature13475.
138. Tanaka K, Hosozawa M, Kudo N, et al. The pilot study: Sphingomyelin-fortified milk has a positive association with the neurobehavioural development of very low birth weight infants during infancy, randomized control trial. Brain Dev 2013;35(1):45–52. DOI: 10.1016/j.braindev.2012.03.004.
139. Tayebati SK, Amenta F. Choline-containing phospholipids: Relevance to brain functional pathways. Clin Chem Lab Med 2013;51(3):513–521. DOI: 10.1515/cclm-2012-0559.
140. Derbyshire E, Obeid R. Choline, neurological development and brain function: A systematic review focusing on the first 1000 days. Nutrients 2020;12(6):1731. DOI: 10.3390/nu12061731.

141. Addis MF, Pisanu S, Ghisaura S, et al. Proteomics and pathway analyses of the milk fat globule in sheep naturally infected by Mycoplasma agalactiae provide indications of the in vivo response of the mammary epithelium to bacterial infection. Infect Immun. 2011;79(9):3833–3845. DOI: 10.1128/IAI.00040-11.
142. Reinhardt TA, Sacco RE, Nonnecke BJ, et al. Bovine milk proteome: Quantitative changes in normal milk exosomes, milk fat globule membranes and whey proteomes resulting from Staphylococcus aureus mastitis. J Proteomics 2013;82:141–154. DOI: 10.1016/j.jprot.2013.02.013.
143. Hewelt-Belka W, Garwolinska D, Mlynarczyk M, et al. Comparative lipidomic study of human milk from different lactation stages and milk formulas. Nutrients 2020;12(7):2165. DOI: 10.3390/nu12072165.
144. Yaron S, Shachar D, Abramas L, et al. Effect of high beta-palmitate content in infant formula on the intestinal microbiota of term infants. J Pediatr Gastroenterol Nutr 2013;56(4):376–381. DOI: 10.1097/MPG.0b013e31827e1ee2.
145. Le Huerou-Luron I, Bouzerzour K, Ferret-Bernard S, et al. A mixture of milk and vegetable lipids in infant formula changes gut digestion, mucosal immunity and microbiota composition in neonatal piglets. Eur J Nutr 2018;57(2):463–476. DOI: 10.1007/s00394-016-1329-3.
146. Liao Y, Alvarado R, Phinney B, et al. Proteomic characterization of human milk fat globule membrane proteins during a 12 month lactation period. J Proteome Res 2011;10(8):3530–3541. DOI: 10.1021/pr200149t.
147. Afrache H, Gouret P, Ainouche S, et al. The butyrophilin (BTN) gene family: From milk fat to the regulation of the immune response. Immunogenetics 2012;64(11):781–794. DOI: 10.1007/s00251-012-0619-z.
148. Ogg SL, Weldon AK, Dobbie L, et al. Expression of butyrophilin (Btn1a1) in lactating mammary gland is essential for the regulated secretion of milk-lipid droplets. Proc Natl Acad Sci USA 2004;101(27):10084–10089. DOI: 10.1073/pnas.0402930101.
149. Chen W, Zhang Z, Zhang S, et al. MUC1: Structure, function, and clinic application in epithelial cancers. Int J Mol Sci 2021;22(12):6567. DOI: 10.3390/ijms22126567.
150. Martin HM, Hancock JT, Salisbury V, et al. Role of xanthine oxidoreductase as an antimicrobial agent. Infect Immun 2004;72(9):4933–4939. DOI: 10.1128/IAI.72.9.4933-4939.2004.
151. Yi YS. Functional role of milk fat globule-epidermal growth factor VIII in macrophage-mediated inflammatory responses and inflammatory/autoimmune diseases. Mediators Inflamm 2016;2016:5628486. DOI: 10.1155/2016/5628486.
152. Xu H, Diolintzi A, Storch J. Fatty acid-binding proteins: Functional understanding and diagnostic implications. Curr Opin Clin Nutr Metab Care 2019;22(6):407–412. DOI: 10.1097/MCO.0000000000000600.
153. Demmelmair H, Prell C, Timby N, et al. Benefits of lactoferrin, osteopontin and milk fat globule membranes for infants. Nutrients 2017;9(8):817. DOI: 10.3390/nu9080817.
154. Liu B, Newburg DS. Human milk glycoproteins protect infants against human pathogens. Breastfeed Med 2013;8(4):354–362. DOI: 10.1089/bfm.2013.0016.
155. Singh SB, Carroll-Portillo A, Coffman C, et al. Intestinal alkaline phosphatase exerts anti-inflammatory effects against lipopolysaccharide by inducing autophagy. Sci Rep 2020;10(1):3107. DOI: 10.1038/s41598-020-59474-6.
156. Kotozaki Y, Satoh M, Nasu T, et al. Human plasma xanthine oxidoreductase activity in cardiovascular disease: Evidence from a population-based study. Biomedicines 2023;11(3):754. DOI: 10.3390/biomedicines11030754.
157. Honvo-Houeto E, Henry C, Chat S, et al. The endoplasmic reticulum and casein-containing vesicles contribute to milk fat globule membrane. Mol Biol Cell 2016;27(19):2946–2964. DOI: 10.1091/mbc.E16-06-0364.
158. Wedekind SIS, Shenker NS. Antiviral properties of human milk. Microorganisms 2021;9(4):715. DOI: 10.3390/microorganisms 9040715.
159. Martin Carli JF, Dzieciatkowska M, Hernandez TL, et al. Comparative proteomic analysis of human milk fat globules and paired membranes and mouse milk fat globules identifies core cellular systems contributing to mammary lipid trafficking and secretion. Front Mol Biosci 2023;10:1259047. DOI: 10.3389/fmolb.2023.1259047.
160. Almeida CC, Mendonca Pereira BF, Leandro KC, et al. Bioactive compounds in infant formula and their effects on infant nutrition and health: A systematic literature review. Int J Food Sci 2021;2021:8850080. DOI: 10.1155/2021/8850080.
161. Zhang Y, Zhang X, Mi L, et al. Comparative proteomic analysis of proteins in breast milk during different lactation periods. Nutrients 2022;14(17):3648. DOI: 10.3390/nu14173648.
162. Janiszewska E, Kmieciak A, Kacperczyk M, et al. The influence of clusterin glycosylation variability on selected pathophysiological processes in the human body. Oxid Med Cell Longev 2022;2022:7657876. DOI: 10.1155/2022/7657876.
163. Melnik BC, Schmitz G. Milk's role as an epigenetic regulator in health and disease. Diseases 2017;5(1):12. DOI: 10.3390/diseases5010012.
164. Carrillo-Lozano E, Sebastian-Valles F, Knott-Torcal C. Circulating microRNAs in breast milk and their potential impact on the infant. Nutrients 2020;12(10):3066. DOI: 10.3390/nu12103066.
165. Innis SM, Dyer R. Dietary triacylglycerols with palmitic acid (16:0) in the 2-position increase 16:0 in the 2-position of plasma and chylomicron triacylglycerols, but reduce phospholipid arachidonic and docosahexaenoic acids, and alter cholesteryl ester metabolism in formula-Fed piglets. J Nutr 1997;127(7):1311–1319. DOI: 10.1093/jn/127.7.1311.
166. Baldi A, Pinotti L. Lipophilic microconstituents of milk. Adv Exp Med Biol 2008;606:109–125. DOI: 10.1007/978-0-387-74087-4_3.
167. Hamosh M. The role of lingual lipase in neonatal fat digestion. Ciba Found Symp 1979(70):69–98. DOI: 10.1002/9780470720530.ch5.
168. Smith LJ, Kaminsky S, D'Souza SW. Neonatal fat digestion and lingual lipase. Acta Paediatr Scand 1986;75(6):913–918. DOI: 10.1111/j.1651-2227.1986.tb10316.x.
169. Lopez-Lopez A, Lopez-Sabater MC, Campoy-Folgoso C, et al. Fatty acid and sn-2 fatty acid composition in human milk from Granada (Spain) and in infant formulas. Eur J Clin Nutr 2002;56(12):1242–1254. DOI: 10.1038/sj.ejcn.1601470.
170. Hamosh M, Scanlon JW, Ganot D, et al. Fat digestion in the newborn. Characterization of lipase in gastric aspirates of premature and term infants. J Clin Invest 1981;67(3):838–846. DOI: 10.1172/jci110101.
171. Freed LM, Berkow SE, Hamosh P, et al. Lipases in human milk: Effect of gestational age and length of lactation on enzyme activity. J Am Coll Nutr 1989;8(2):143–150. DOI: 10.1080/07315724.1989.10720289.
172. Ye A, Cui J, Singh H. Proteolysis of milk fat globule membrane proteins during in vitro gastric digestion of milk. J Dairy Sci 2011;94(6):2762–2770. DOI: 10.3168/jds.2010-4099.
173. Holton TA, Vijayakumar V, Dallas DC, et al. Following the digestion of milk proteins from mother to baby. J Proteome Res 2014;13(12):5777–5783. DOI: 10.1021/pr5006907.
174. Bourlieu C, Mahdoueni W, Paboeuf G, et al. Physico-chemical behaviors of human and bovine milk membrane extracts and their influence on gastric lipase adsorption. Biochimie 2020;169:95–105. DOI: 10.1016/j.biochi.2019.12.003.
175. Li X, Lindquist S, Lowe M, et al. Bile salt-stimulated lipase and pancreatic lipase-related protein 2 are the dominating lipases in neonatal fat digestion in mice and rats. Pediatr Res 2007;62(5):537–541. DOI: 10.1203/PDR.0b013e3181559e75.
176. Xiao X, Mukherjee A, Ross LE, et al. Pancreatic lipase-related protein-2 (PLRP2) can contribute to dietary fat digestion in human newborns. J Biol Chem 2011;286(30):26353–26363. DOI: 10.1074/jbc.M111.249813.
177. Nilsson A, Duan RD, Ohlsson L. Digestion and absorption of milk phospholipids in newborns and adults. Front Nutr 2021;8:724006. DOI: 10.3389/fnut.2021.724006.
178. Lindquist S, Hernell O. Lipid digestion and absorption in early life: An update. Curr Opin Clin Nutr Metab Care 2010;13(3):314–320. DOI: 10.1097/MCO.0b013e328337bbf0.

179. Hernell O, Blackberg L. Human milk bile salt-stimulated lipase: Functional and molecular aspects. J Pediatr 1994;125(5 Pt 2):S56–S61. DOI: 10.1016/s0022-3476(06)80737-7.
180. Duan RD. Alkaline sphingomyelinase: An old enzyme with novel implications. Biochim Biophys Acta 2006;1761(3):281–291. DOI: 10.1016/j.bbalip.2006.03.007.
181. Duan RD, Cheng Y, Jonsson BA, et al. Human meconium contains significant amounts of alkaline sphingomyelinase, neutral ceramidase, and sphingolipid metabolites. Pediatr Res 2007;61(1): 61–66. DOI: 10.1203/01.pdr.0000250534.92934.c2.
182. Nilsson A. Role of sphingolipids in infant gut health and immunity. J Pediatr 2016;173(Suppl):S53–S69. DOI: 10.1016/j.jpeds.2016.02.076.
183. Ahmed TB, Eggesbo M, Criswell R, et al. Total fatty acid and polar lipid species composition of human milk. Nutrients 2021;14(1):158. DOI: 10.3390/nu14010158.
184. Innis SM, Dyer R, Quinlan P, et al. Palmitic acid is absorbed as sn-2 monopalmitin from milk and formula with rearranged triacylglycerols and results in increased plasma triglyceride sn-2 and cholesteryl ester palmitate in piglets. J Nutr 1995;125(1):73–81. DOI: 10.1093/jn/125.1.73.
185. Abrahamse E, Minekus M, van Aken GA, et al. Development of the digestive system-experimental challenges and approaches of infant lipid digestion. Food Dig 2012;3(1–3):63–77. DOI: 10.1007/s13228-012-0025-x.
186. Blackberg L, Hernell O, Olivecrona T. Hydrolysis of human milk fat globules by pancreatic lipase: Role of colipase, phospholipase A2, and bile salts. J Clin Invest 1981;67(6):1748–1752. DOI: 10.1172/jci110213.
187. Lu N, Wang J, Chen Z, et al. The effect of adding phospholipids before homogenization on the properties of milk fat globules. Food Sci Tech 2021;146:111659. DOI: 10.1016/j.lwt.2021.111659.
188. Oosting A, Kegler D, Wopereis HJ, et al. Size and phospholipid coating of lipid droplets in the diet of young mice modify body fat accumulation in adulthood. Pediatr Res 2012;72(4):362–369. DOI: 10.1038/pr.2012.101.
189. Timby N, Domellof E, Hernell O, et al. Neurodevelopment, nutrition, and growth until 12 mo of age in infants fed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: A randomized controlled trial. Am J Clin Nutr 2014;99(4):860–868. DOI: 10.3945/ajcn.113.064295.
190. Timby N, Adamsson M, Domellof E, et al. Neurodevelopment and growth until 6.5 years of infants who consumed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: A randomized controlled trial. Am J Clin Nutr 2021;113(3):586–592. DOI: 10.1093/ajcn/nqaa354.
191. Nieto-Ruiz A, Dieguez E, Sepulveda-Valbuena N, et al. The effects of an infant formula enriched with milk fat globule membrane, long-chain polyunsaturated fatty acids and synbiotics on child behavior up to 2.5 years old: The COGNIS study. Nutrients 2020;12(12). DOI: 10.3390/nu12123825.
192. Michalski M, Michel F, Sainmont D, et al. Apparent ζ-potential as a tool to assess mechanical damages to the milk fat globule membrane, colloids and surfaces B: Biointerfaces. J Col Surfb 2002;23(1):23–30. DOI: 10.1016/S0927-7765(01)00203-X.
193. Cebo C, Lopez C, Henry C, et al. Goat alpha(s1)-casein genotype affects milk fat globule physicochemical properties and the composition of the milk fat globule membrane. J Dairy Sci 2012;95(11):6215–6229. DOI: 10.3168/jds.2011-5233.
194. Aguilera JM. The food matrix: Implications in processing, nutrition and health. Crit Rev Food Sci Nutr 2019;59(22):3612–3629. DOI: 10.1080/10408398.2018.1502743.
195. Vandenplas Y, Ksiazyk J, Luna MS, et al. Partial hydrolyzed protein as a protein source for infant feeding: Do or don't? Nutrients 2022;14(9):1720. DOI: 10.3390/nu14091720.
196. Guo Q, Bellissimo N, Rousseau D. The physical state of emulsified edible oil modulates its in vitro digestion. J Agric Food Chem 2017;65(41):9120–9127. DOI: 10.1021/acs.jafc.7b03368.
197. Li F, Wu SS, Berseth CL, et al. Improved neurodevelopmental outcomes associated with bovine milk fat globule membrane and lactoferrin in infant formula: A randomized, controlled trial. J Pediatr 2019;215:24–31.e8. DOI: 10.1016/j.jpeds.2019.08.030.
198. Schneider N, Hauser J, Oliveira M, et al. Sphingomyelin in brain and cognitive development: Preliminary data. eNeuro 2019;6(4). DOI: 10.1523/ENEURO.0421-18.2019.
199. Zheng L, Fleith M, Giuffrida F, et al. Dietary polar lipids and cognitive development: A narrative review. Adv Nutr 2019;10(6):1163–1176. DOI: 10.1093/advances/nmz051.
200. Bitman J, Wood DL, Mehta NR, et al. Comparison of the phospholipid composition of breast milk from mothers of term and preterm infants during lactation. Am J Clin Nutr 1984;40(5):1103–1119. DOI: 10.1093/ajcn/40.5.1103.
201. Li T, Gong H, Yuan Q, et al. Supplementation of polar lipids-enriched milk fat globule membrane in high-fat diet-fed rates during pregnancy and lactation promotes brown/beige adipocyte development and prevents obesity in male offspring. FASEB J 2020;34(3):4619–4634. DOI: 10.1096/fj.201901867RRR.
202. Zhang Q, Ye L, Xin F, et al. Milk fat globule membrane supplementation during suckling ameliorates maternal high fat diet-induced hepatic steatosis in adult male offspring of mice. J Nutr 2021;151(6):1487–1496. DOI: 10.1093/jn/nxab026.
203. Han L, Du M, Ren F, et al. Milk polar lipids supplementation to obese rats during pregnancy and lactation benefited skeletal outcomes of male offspring. Mol Nutr Food Res 2021;65(15):e2001208. DOI: 10.1002/mnfr.202001208.
204. He X, Parenti M, Grip T, et al. Fecal microbiome and metabolome of infants fed bovine MFGM supplemented formula or standard formula with breast-fed infants as reference: A randomized controlled trial. Sci Rep 2019;9(1):11589. DOI: 10.1038/s41598-019-47953-4.
205. O'Mahony SM, McVey Neufeld KA, Waworuntu RV, et al. The enduring effects of early-life stress on the microbiota-gut-brain axis are buffered by dietary supplementation with milk fat globule membrane and a prebiotic blend. Eur J Neurosci 2020;51(4): 1042–1058. DOI: 10.1111/ejn.14514.
206. Kosmerl E, Rocha-Mendoza D, Ortega-Anaya J, et al. Improving human health with milk fat globule membrane, lactic acid bacteria, and bifidobacteria. Microorganisms 2021;9(2). DOI: 10.3390/microorganisms9020341.
207. Charton E, Bourgeois A, Bellanger A, et al. Infant nutrition affects the microbiota-gut-brain axis: Comparison of human milk vs. infant formula feeding in the piglet model. Front Nutr 2022;9:976042. DOI: 10.3389/fnut.2022.976042.
208. Wu Y, Zhang X, Han D, et al. Early life administration of milk fat globule membrane promoted SCFA-producing bacteria colonization, intestinal barriers and growth performance of neonatal piglets. Anim Nutr 2021;7(2):346–355. DOI: 10.1016/j.aninu.2020.07.012.
209. Zhao J, Yi W, Liu B, et al. MFGM components promote gut Bifidobacterium growth in infant and in vitro. Eur J Nutr 2022;61(1): 277–288. DOI: 10.1007/s00394-021-02638-5.
210. Yu Z, Huang S, Li Y, et al. Milk fat globule membrane alleviates short bowel syndrome-associated liver injury in rats through inhibiting autophagy and NLRP3 inflammasome activation. Front Nutr 2022;9:758762. DOI: 10.3389/fnut.2022.758762.
211. Zheng D, Kern L, Elinav E. The NLRP6 inflammasome. Immunology 2021;162(3):281–289. DOI: 10.1111/imm.13293.
212. Belizario JE, Faintuch J. Microbiome and gut dysbiosis. Exp Suppl 2018;109:459–476. DOI: 10.1007/978-3-319-74932-7_13.
213. Levy M, Thaiss CA, Zeevi D, et al. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. Cell 2015;163(6):1428–1443. DOI: 10.1016/j.cell.2015.10.048.
214. Zhang L, Chichlowski M, Gross G, et al. Milk fat globule membrane protects lactobacillus rhamnosus gg from bile stress by regulating exopolysaccharide production and biofilm formation. J Agric Food Chem 2020;68(24):6646–6655. DOI: 10.1021/acs.jafc.0c02267.
215. Jiang C, Cheong LZ, Zhang X, et al. Dietary sphingomyelin metabolism and roles in gut health and cognitive development. Adv Nutr 2022;13(2):474–491. DOI: 10.1093/advances/nmab117.

216. Narita T, Naganuma T, Sase Y, et al. Long-chain bases of sphingolipids are transported into cells via the acyl-CoA synthetases. Sci Rep 2016;6:25469. DOI: 10.1038/srep25469.
217. Xiong Y, Hla T. S1P control of endothelial integrity. Curr Top Microbiol Immunol 2014;378:85–105. DOI: 10.1007/978-3-319-05879-5_4.
218. Rohrhofer J, Zwirzitz B, Selberherr E, et al. The impact of dietary sphingolipids on intestinal microbiota and gastrointestinal immune homeostasis. Front Immunol 2021;12:635704. DOI: 10.3389/fimmu.2021.635704.
219. Fontecha J, Brink L, Wu S, et al. Sources, production, and clinical treatments of milk fat globule membrane for infant nutrition and well-being. Nutrients 2020;12(6). DOI: 10.3390/nu12061607.
220. Billeaud C, Puccio G, Saliba E, et al. Safety and tolerance evaluation of milk fat globule membrane-enriched infant formulas: A randomized controlled multicenter non-inferiority trial in healthy term infants. Clin Med Insights Pediatr 2014;8:51–60. DOI: 10.4137/CMPed.S16962.
221. Bhinder G, Allaire JM, Garcia C, et al. Milk fat globule membrane supplementation in formula modulates the neonatal gut microbiome and normalizes intestinal development. Sci Rep 2017;7:45274. DOI: 10.1038/srep45274.
222. Suarez-Martinez C, Santaella-Pascual M, Yague-Guirao G, et al. Infant gut microbiota colonization: Influence of prenatal and postnatal factors, focusing on diet. Front Microbiol 2023;14:1236254. DOI: 10.3389/fmicb.2023.1236254.
223. Rueda R, Sabatel JL, Maldonado J, et al. Addition of gangliosides to an adapted milk formula modifies levels of fecal Escherichia coli in preterm newborn infants. J Pediatr 1998;133(1):90–94. DOI: 10.1016/s0022-3476(98)70184-2.
224. Pellegrini A, Thomas U, Bramaz N, et al. Isolation and identification of three bactericidal domains in the bovine alpha-lactalbumin molecule. Biochim Biophys Acta 1999;1426(3):439–448. DOI: 10.1016/s0304-4165(98)00165-2.
225. Nakimbugwe D, Masschalck B, Deckers D, et al. Cell wall substrate specificity of six different lysozymes and lysozyme inhibitory activity of bacterial extracts. FEMS Microbiol Lett 2006;259(1):41–46. DOI: 10.1111/j.1574-6968.2006.00240.x.
226. Liu B, Yu Z, Chen C, et al. Human milk mucin 1 and mucin 4 inhibit Salmonella enterica serovar typhimurium invasion of human intestinal epithelial cells in vitro. J Nutr 2012;142(8):1504–1509. DOI: 10.3945/jn.111.155614.
227. Yolken RH, Peterson JA, Vonderfecht SL, et al. Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis. J Clin Invest 1992;90(5):1984–1991. DOI: 10.1172/JCI116078.
228. Bezirtzoglou E, Stavropoulou E. Immunology and probiotic impact of the newborn and young children intestinal microflora. Anaerobe 2011;17(6):369–374. DOI: 10.1016/j.anaerobe.2011.03.010.
229. Bintsis T. Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. AIMS Microbiol 2018;4(4):665–684. DOI: 10.3934/microbiol.2018.4.665.
230. Pacheco AR, Barile D, Underwood MA, et al. The impact of the milk glycobiome on the neonate gut microbiota. Annu Rev Anim Biosci 2015;3:419–445. DOI: 10.1146/annurev-animal-022114-111112.
231. La Reau AJ, Suen G. The Ruminococci: Key symbionts of the gut ecosystem. J Microbiol 2018;56(3):199–208. DOI: 10.1007/s12275-018-8024-4.
232. Martinez-Sanchez V, Visitacion Calvo M, Viera I, et al. Mechanisms for the interaction of the milk fat globule membrane with the plasma membrane of gut epithelial cells. Food Res Int 2023;173(Pt 1):113330. DOI: 10.1016/j.foodres.2023.113330.
233. O'Brien K, Breyne K, Ughetto S, et al. RNA delivery by extracellular vesicles in mammalian cells and its applications. Nat Rev Mol Cell Biol 2020;21(10):585–606. DOI: 10.1038/s41580-020-0251-y.

REVIEW ARTICLE

Hemostasis Assessment in Neonates: Evaluation of Viscoelastic Properties of Blood Clots

Brunetta Guaragni[1], Mario Motta[2]

Received on: 04 February 2024; *Accepted on*: 03 March 2024; *Published on*: 26 March 2024

Abstract

In recent years, a new approach to neonatal hemostasis has been taking hold. The term "developmental hemostasis" refers to the dynamic, age-related physiological changes seen in the hemostatic system in neonates and young infants. Most conventional coagulation tests have limitations as these are focused primarily on the procoagulant factors and do not inform about platelet function and the levels/activity of von Willebrand factor (vWF), natural anticoagulants, and fibrinolytic activity. In this scheme, viscoelastic coagulation tests can rapidly provide a potentially useful, panoramic assessment of the entire coagulation process from the formation to degradation of clots, platelet function, and fibrinolysis. This is a narrative review on the use of viscoelastic tests in neonatal care; we have included information from our own clinical experience and from an extensive literature search spanning PubMed, Scopus, and Web of Science. This review is important because tests can help identify premature/critically ill infants who may be at risk of hemorrhage during routine care or after surgery and may need corrective transfusions with appropriate blood products.

Keywords: Activated partial prothrombin time, Developmental hemostasis, Fibrinogen levels, Fibrinolytic activity, Natural anticoagulants, Platelet function, Procoagulant factors, Prothrombin time, Viscoelastic coagulation tests, von Willebrand factor.

Newborn (2024): 10.5005/jp-journals-11002-0089

Highlights

- In recent years, a new approach to neonatal hemostasis, called "developmental hemostasis" has been recognized. It refers to the dynamic, age-related physiological changes seen in the hemostatic system in neonates and young infants.
- Most conventional coagulation tests have limitations as these are focused primarily on the procoagulant factors and do not inform about platelet function, von Willebrand factor (vWF), natural anticoagulants, and fibrinolytic activity.
- Viscoelastic coagulation tests, such as thromboelastography (TEG) and thromboelastometry, can provide a rapid, global assessment of clotting in a graphical trace of the entire coagulation process from clot formation to clot degradation, platelet function, and fibrinolysis.
- The viscoelastic coagulation tests involve the positioning of a whole blood sample with a rotating activator in a cup with a pin at its center. A fibrin strand forms and slows down further rotation and the blood slowly coagulates.
- Preliminary studies show that viscoelastic tests may help overcome the limitations of conventional coagulation tests; there are advantages such as bedside testing, a panoramic view of the entire hemostatic process, and the need for minimal blood volumes.

Introduction

Bleeding and thrombosis are seen frequently in premature and critically ill infants. Historically, the diagnosis of coagulation disorders in neonates included platelet counts and standard coagulation tests such as the measurement of prothrombin time (PT), activated partial prothrombin time (aPTT), and fibrinogen levels. These tests measure plasma concentrations and activity of procoagulant proteins but do not provide detailed information about platelet function, concentrations of the vWF and natural anticoagulants, and fibrinolysis. In the absence of these data, we are not able to obtain a useful panoramic view of the entire hemostatic system.[1] These measurements are particularly important in infants as the hemostatic function is a dynamic system that undergoes quantitative/qualitative changes during development and postnatal age, and also gets disrupted during critical illness. Clearly, conventional coagulation tests may not be adequate to identify inherited/acquired coagulation disorders of various degrees of severity, and to guide transfusions frequently needed in premature/critically ill neonates.[2]

Viscoelastic coagulation tests, such as TEG and rotational thromboelastometry (ROTEM), may help overcome some of these limitations. These testing methods can provide a rapid, global assessment of clotting in a graphical trace of the entire coagulation process from clot formation to clot degradation, which indirectly also informs about platelet function and fibrinolysis. In this way, the tests provide a better assessment of the *in vivo* conditions than conventional coagulation tests.[3] In this article, we aim to provide an overview of current, evidence-based information on

[1]Department of Neonatal Intensive Care Unit, Children's Hospital ASST – Spedali Civili, Brescia, Italy

[2]Department of Neonatology and Neonatal Intensive Care, AUSL di Bologna, Bologna, Italy

Corresponding Author: Mario Motta, Department of Neonatology and Neonatal Intensive Care, AUSL di Bologna, Bologna, Italy, Phone: +390516478563, e-mail: mario.motta@ausl.bologna.it

How to cite this article: Guaragni B, Motta M. Hemostasis Assessment in Neonates: Evaluation of Viscoelastic Properties of Blood Clots. Newborn 2024;3(1):38–44.

Source of support: Nil

Conflict of interest: None

the clinical applications of viscoelastic testing in the context of neonatal intensive care. In these patients, rapid assessment of the coagulation systems(s) is frequently needed to determine the risk of bleeding in critical organ systems, the need for clinical and laboratory-based monitoring, and to guide blood product administration and therapy.

From "Developmental Hemostasis" to Understanding the Limits of Standard Coagulation Tests

In premature/critically ill infants in neonatal intensive care units (NICUs), there is a need to understand physiological hemostatic factors/processes and the alterations in various disease states. This information is needed not only to understand the risk of localized/systemic hemorrhage but also to guide transfusion management in these patients. The term "developmental hemostasis" was first coined by Maureen Andrews to describe the dynamic evolution of the hemostatic system during childhood.[4] The hemostatic system undergoes an age-dependent dynamic evolution during which plasma levels/isotypes of most proteins in the coagulation pathways change significantly with age. Neonates have lower plasma concentrations of vitamin K-dependent coagulation factors and contact factors than adults. During the first 6 months of life, the concentration of these coagulation factors rises gradually to reach levels seen in adults. In contrast, plasma levels of fibrinogen, factor V (FV), FVIII, FXIII, and the vWF resemble those in adults. In the natural inhibitor system, plasma concentrations of antithrombin, protein C and protein S are lower at birth and reach adult levels at about 6 months. These differences in the developmental trajectories of pro- and anticoagulant proteins are seen in both pre- and full-term infants; these changes comprise a regularly seen dynamic equilibrium in good infants.[5]

Age-related changes in clotting proteins result in corresponding changes in standard coagulation tests such as PT and aPTT. Considering the age-dependent specificity of hemostasis, the evaluation, and the interpretation of coagulation tests in newborns need to be based on appropriate reference ranges for gestational and postnatal age.[6–8] Both PT and aPTT are longer in infants than in adults, possibly due to lower levels of vitamin K-dependent factors. Consequently, these measurements are not as useful in the evaluation of acquired neonatal coagulopathies unless we have "control" results that were obtained prior to the onset of illness. Furthermore, these data do not include the effects of platelet function and the levels of vWF, natural anticoagulants, and fibrinolytic activity, without which the analysis of overall hemostasis remains incomplete.[9]

In neonates, the results of coagulation tests have been viewed as atypical/abnormal as these differ from those in adults, and have not been accurate predictors of bleeding and clinical severity of the underlying disease.[8,10–12] Therefore, routine coagulation testing is no longer performed in most NICUs at admission; most studies showed that such testing only increased the rates of transfusions without convincing evidence of benefit. Furthermore, these tests required relatively large volumes of blood (0.8–2 mL) and contributed to iatrogenic anemia, especially in very low birth weight (VLBW) infants.[13] Finally, in the context of acute, major hemorrhage the length of time required to obtain results also became an important limitation.

Viscoelastic Coagulation Tests

Given the complexity of the coagulation process and the inadequacy of standard laboratory tests, nowadays, the evaluation with viscoelastic coagulation tests is a better, rapid, panoramic assessment of hemostasis, which provides information about clot strength, dynamics, and breakdown. With information about the viscoelastic properties of the clot, these data differ from conventional coagulation tests as these also inform about the entire coagulation process, including clot development, stabilization, and dissolution; the interaction between plasma coagulation proteins; and qualitative/quantitative information about platelets and blood cells. Not surprisingly, the results are a better reflection of the situation *in vivo* than the standard tests. The interest and experience with the viscoelastic tests in neonates have been increasing over the years and its use seems promising in the diagnosis and treatment of acquired coagulopathies.[14,15] Thromboelastography (TEG) (TEG®, Haemonetics, Braintree, Massachusetts, USA) and ROTEM (TEM international, Munich, Germany) have been the main providers so far, although other similar/comparable devices are now also available.

The original TEG and ROTEM technologies involve the positioning of a whole blood sample with an activator in a cup with a pin at its center. With TEG, the cup rotates, whereas the pin moves around a circulation axis in the ROTEM. A fibrin strand forms, making the pin adhere to the wall of the cup. This slows down further rotation and the blood slowly coagulates. Blood clotting leads to the development of the TEG/ROTEM trace; the interpretations are similar, but not identical, to other different and non-interchangeable reference range parameters.[1]

Several newer cartridge-based devices have improved the viscoelastic assessment as these do not need controlled pipetting, which is subject to interuser modifications and consequent errors. One of these is the viscoelastic coagulation monitor (VCM) device (VCM™, Entegrion, Inc., Durham, North Carolina, USA), which provides an analysis of the coagulation state in approximately 1 hour with 300 μL of a fresh, whole blood sample. This device is quick and easy to use, is portable, and has the potential for more widespread clinical use in neonatal intensive care (Fig. 1).[16]

The following parameters of clot formation and lysis are evaluated as a representation of hemostasis:

- Clotting time (CT) (reaction rate (R) in minutes/CT). The time, expressed in minutes, reaches 2-mm amplitude in TEG and ROTEM, 1% above the baseline in VCM. It represents the speed of fibrin formation;
- Clot kinetics [kinetics (*K*) in seconds/clot formation time (CFT)]. Time, expressed in minutes, for clot amplitude to reach from 2 to 20 mm in TEG/ROTEM, from 1 to 10% amplitude in VCM. This time is a measure of the clot kinetics to reach a fixed level of clot strength. It correlates with fibrinogen level and, to a lesser extent, with both platelet function and values;
- Alpha angle (α). The angle formed between the midline and the tangent to the main body of the trace. Like *K*, α correlates with fibrinogen level, with platelet function and values;
- Maximum amplitude [MA/maximum clot firmness (MCF)]. The amplitude at the widest point of the trace represents the maximum clot strength as the result of the modest contribution of fibrin and the much more significant contribution of platelets;
- Fibrinolysis [clot lysis at 30 minutes after maximum clot strength (LY30)]. Reduction in amplitude 30 minutes after MA/MCF. It is expressed as a percentage of MA/MCF.[17]

One of the main advantages of viscoelastic coagulation tests is the need for smaller amounts of blood, 340 vs 800–2000 μL that was needed to perform conventional coagulation tests. Furthermore, blood samples can be obtained from a peripheral vein or artery or

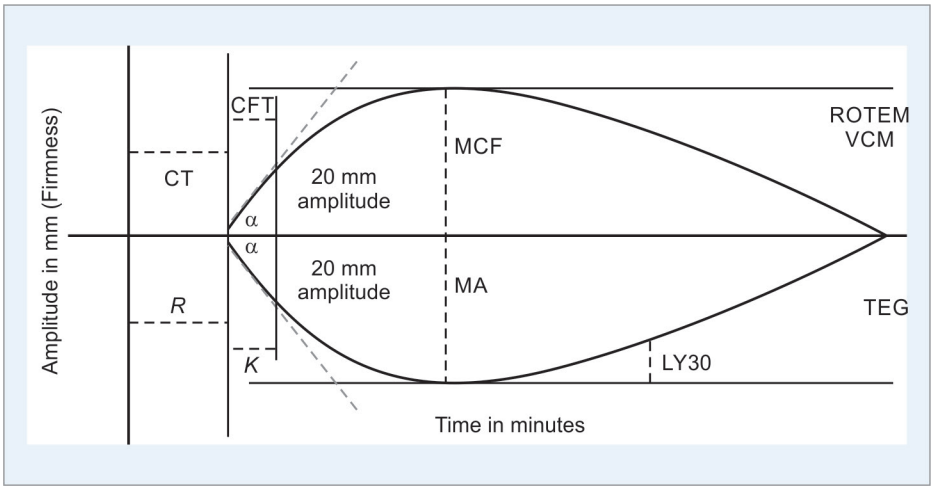


Fig. 1: Schematic representation of normal TEG/ROTEM/VCM traces

via a heel stick, maintaining both feasibility and reproducibility of the test results.[18] Another advantage of viscoelastic coagulation tests is the real-time evaluation of the hemostatic state as the results become available in just about 20 minutes, compared to the 40–90 minutes that were needed for conventional tests. Finally, these tests can be performed at the patient's bedside, allowing quick, individualized, and targeted therapeutic interventions.[9]

Reference Ranges for Viscoelastic Coagulation Tests

Many published studies are available proving the reproducibility and reliability of the viscoelastic coagulation tests to assess hemostasis in the neonatal population.[19,20] Motta et al.[21] evaluated TEG parameters in healthy preterm infants and developed reference intervals. The TEG parameters between early-preterm (<32 weeks' gestation) resembled those in moderate-/late preterm (from 32 to less than 37 weeks' gestation) neonates, suggesting that hemostatic function matures at birth even in very premature infants.[21] In another study, Sewell et al. established normative TEG ranges for blood samples from term infants obtained in tubes containing citrate or heparinase.[22] In an observational study, Ghirardello et al. performed duplicate TEG measurements on blood samples obtained from VLBW neonates and found an acceptable level of agreement between these duplicates. These data confirmed the reliability of TEG to assess hemostasis in neonates.[19]

In another study, Sokou et al. established reference ranges for peripheral arterial whole blood EXTEMROTEM® assay in pre- and full-term neonates; the two groups showed comparable results. Preterm neonates showed enhanced fibrinolytic activity as follows: Lysis at 60 minutes (LY60) values were significantly lower than full-term neonates ($p = 0.006$). There was also a significant correlation between lysis at 60 minutes (LY60) with gestational age and birth weight in preterm infants.[23]

The same author compared clot formation in neonates with small-for-gestational-age neonates' weights with those who were born appropriate-for-gestational-age weight (AGA) using EXTEM ROTEM® analysis. The two groups showed comparable results.[24]

Viscoelastic Coagulation Tests in Acquired Coagulation Disorders of Newborns

Several studies have now proved the reproducibility and reliability of the viscoelastic coagulation tests to assess hemostasis in the neonatal population by developing local, age-, analyzer-, and reagent-appropriate reference ranges. We also now have data for viscoelastic test parameters to evaluate the correlation between coagulopathy and clinical bleeding.

Radicioni et al. measured the thromboelastographic profiles of 49 premature neonates with and without intracranial hemorrhage (ICH).[15] No significant differences in TEG and standard coagulation test values between the two groups were observed at birth. Similar to traditional coagulation tests, TEG assessment failed to identify the underlying coagulopathy of preterm infants with ICH. Interestingly, newborns with ICH showed increased TEG-defined thrombin generation from birth to 21 days of life, suggesting a state of hypercoagulability. It is plausible that TEG cannot detect the bleeding risk of preterm infants, but the presence of hemorrhages such as ICH could be suggested by altered TEGs.

A retrospective study evaluated TEG parameters in healthy preterm neonates and organized the data into reference intervals. The authors also compared 24 preterm infants with active bleeding with 94 who did not have active hemorrhage; the TEG parameters were similar.[21] The bleeding group included 91.6% of preterm neonates with ICH, showing again that preterm infants with or without ICH have similar TEG parameters. In contrast, the normative ranges for citrated-modified and heparinase-modified TEG parameters in term neonates presented by Sewell et al. showed clear thresholds in TEG parameters that were associated with clinical bleeding.[22] Their bleeding group was comprised of neonates with hypoxic–ischemic encephalopathy, respiratory failure requiring extracorporeal membrane oxygenation (ECMO), critical congenital heart disease, and disseminated herpes simplex virus, and therefore, they could not exclude whether any of these illnesses could have affected the TEG parameters.

Forman et al. examined a cohort of 24 infants receiving therapeutic hypothermia for perinatal asphyxia.[25] The TEG assays were simultaneously performed at 33.5 and 37°C for comparison. Also, TEG results were affected by temperature, indicating lower temperatures impaired the coagulation pathways. The TEG parameters predicted clinical bleeding with temperature-dependent thresholds, whereas the standard coagulation tests did not. The authors concluded that TEG results should be carried out under temperature-regulated conditions for neonates undergoing therapeutic hypothermia; if so, this might be a suitable method to

assess the risk of bleeding and optimize transfusion treatment in this population.

In 1995, Stammer et al. used viscoelastography to evaluate the coagulation status of 17 neonates undergoing ECMO for severe respiratory dysfunction.[26] The TEG profiles reflected hemostatic conditions that ranged from severe disseminated intravascular coagulopathy (DIC) to hypercoagulability, identifying clotting abnormalities in 46.5% of the cases. Most were related to platelet dysfunction. The TEG profiles were normal in 73.2% of infants who did not have hemorrhages, whereas only 40% of those with hemorrhage had comparable normal values. In another study, Phillips et al. retrospectively reviewed 46 neonates with congenital diaphragmatic hernia (CDH) between 2008 and 2018 while they were being supported by (ECMO).[27] From 2015, they had implemented and standardized their anticoagulation management by including routine TEG monitoring and found that this implementation improved goal-directed blood product transfusion in neonates with CDH supported by ECMO. Thromboelastography derangements led to a significant increase in platelet transfusions, and fewer cryoprecipitate administration, but no change in the use of fresh frozen plasma (FFP). Additionally, comparing the pre-2015 and the post-2015 groups, they found a significant reduction in the incidence of hemothorax. Both these studies highlight that in neonates undergoing ECMO, the TEG assay can be a useful tool for rapid diagnosis of hemorrhagic conditions and timely administration of blood transfusion products. Peterson et al.[28] examined blood samples from 44 neonates undergoing cardiopulmonary bypass (CPB) to evaluate the heparin–protamine balance and determine its association with postoperative bleeding. They used calibrated automated thrombography, thrombin-initiated fibrin clot kinetic assay (TFCK), aPTT, anti-FXa activity, and thromboelastography. About 36% of their patients had excessive postoperative bleeding. They found that aPTT correlated strongly with TFCK but so much with the anti-FXa and ROTEM assays. This correlation between aPTT and TFCK could have been due to similarities in the measurement of fibrin formation as the endpoint for both assays. None of the coagulation tests predicted postoperative bleeding in these neonates, suggesting that hemorrhagic events were likely multifactorial.[28] In a different single-center observational study, Sokou et al.[29] compared thrombolelastometry parameters in pre- and full-term neonates with confirmed vs suspected sepsis. Septic neonates had a hypocoagulable state, not those with unconfirmed infections. This hypocoagulable profile was more evident in septic neonates with clinically evident bleeding than in those without. They also noted that EXTEM A10 was a strong predictor for bleeding events in this neonatal population.[29]

The same investigators assessed 332 critically ill pre- and full-term neonates to develop a predictive model for bleeding and then internally validated their findings.[30] They performed thromboelastometry and used the neonatal bleeding assessment tool (NeoBAT) to record hemorrhagic events occurring within 24 hours of the ROTEM testing.[31] Thromboelastometry parameters, platelet counts, and creatinine levels were identified as the most robust predictors of hemorrhage and were included in a neonatal bleeding risk (NeoBRis) index. This multivariable prediction model showed excellent performance, suggesting that, after external validation, this could help clinicians to assess the 24-hour bleeding risk and individualize the need for transfusions.

In a recent study, Raffaeli et al.[32] assessed the impact of TEG and VCM implementation on FFP transfusion of neonates undergoing surgery. They found that the TEG and VCM-based quality improvement project improved the hemostatic management of surgical newborns by reducing intraoperative FFP administration. Indeed, neonates presenting prolonged PT or aPTT and normal TEG or VCM parameters did not receive FFP transfusion; the attending physician was probably reassured by a normal viscoelastic trace.

Viscoelastic Coagulation Tests in Congenital Coagulation Disorders of Newborns

In the last few years, viscoelastic coagulation tests have been studied in the diagnosis and management of inherited bleeding disorders, including hemophilia.[33] Standard coagulation tests and factor assays have been used routinely in the diagnosis and management of hemophilia but have some limitations. Both PT and PTT only give information on the initiation of coagulation and aPTT based FVIII:C (factor VIII activity) test does not necessarily correlate with clinical severity.[34] Thus, viscoelastic coagulation tests have been studied to complement standard assays and overcome challenges in hemophilia care.

One of the main challenges in neonates with congenital bleeding disorders is monitoring for routine FVIII prophylaxis as well as a clinical response to different forms of therapy. Hawaj et al.[35] studied 8 severe hemophilia A patients with various bleeding phenotypes after receiving their standard rFVIII prophylaxis dose. Parameters including FVIII:C, TEG, and TGA were monitored over 48 hours. A significant correlation was found between FVIII:C, TEG R-time, and aPTT. At 24 hours, the TEG parameters were subtherapeutic despite the median FVIII:C of 13.0 IU/dL^{1} and lost sensitivity at 48 hours.[35]

In another study, Aghighi et al.[36] demonstrated that measurement of maximum velocity parameters by ROTEM had a 92% sensitivity and 95% specificity for diagnosing hemophilia; these findings correlated strongly with factor VIII levels.[36] Patients with hemophilia are usually classified into mild (FVIII > 5%), moderate (FVIII, 1–5%), and severe (FVIII <1%) groups.[37] One of the main issues is the differentiation of the clinical phenotype in this disease. Patients with severe hemophilia can manifest a mild clinical phenotype and, vice versa.[38] Viscoelastic testing can help differentiate the clinical phenotypes. Ramiz et al.[39] evaluated TEG analysis in patients with severe hemophilia A, who had similar levels of FVIII (<1%) but different coagulation patterns. Patients with milder phenotypes showed a shorter *R*-time and steeper α-angle as a better clot formation. They also used TEG, alongside standard coagulation tests, to monitor routine FVIII prophylaxis and to assess a variable half-life response to F VIII administrations. At 48 hours after infusion, TEG analysis showed a sufficient clotting pattern and led to a less frequent infusion schedule.[39]

In our experience, we have been using viscoelastic tests, TEG 5000 (TEG®, Haemonetics, Braintree, Massachusetts, USA) and VCM (VCM™, Entegrion, Inc., Durham, North Carolina, USA), to assess whole blood hemostasis in acquired and congenital clotting disorders. We followed up with TEG 5000 a term newborn, born by cesarean section with an antenatal diagnosis of bladder exstrophy and hemophilia A. The analysis of the chorionic villi highlighted the presence of a genetic defect in hemizygosis c.–257T>G. On the first day, factor VIII was 12%, defining mild hemophilia. On day 4, the patient underwent surgery for repair and received rFVIII prophylaxis dose. Thromboelastography tracing (Fig. 2), before receiving rFVIII, showed a long *R*- and *K*-time, and a narrow α-angle, while TEG tracing (Fig. 3), after rFVIII administration, depicted a significant

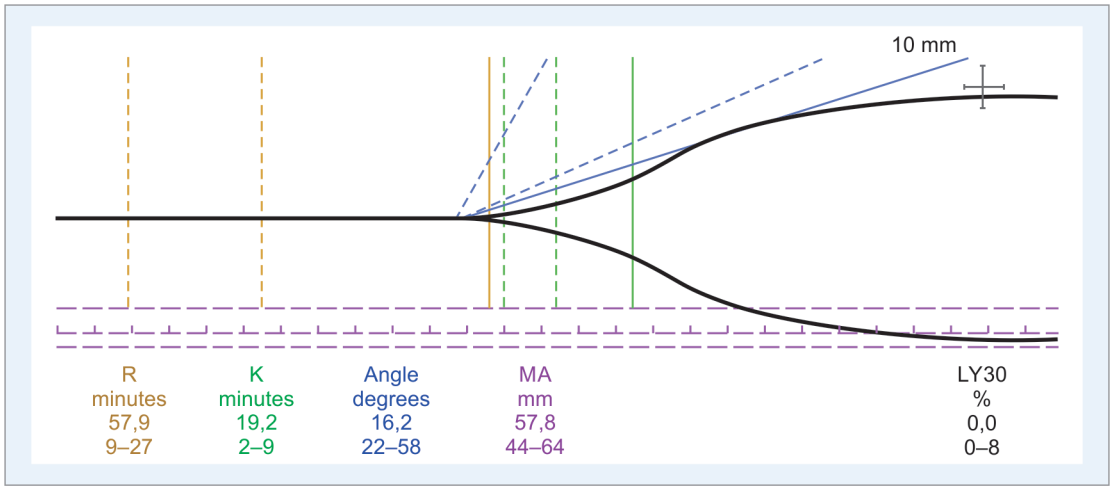


Fig. 2: The trace shows a long *R*- and *K*-time, and a narrow α-angle

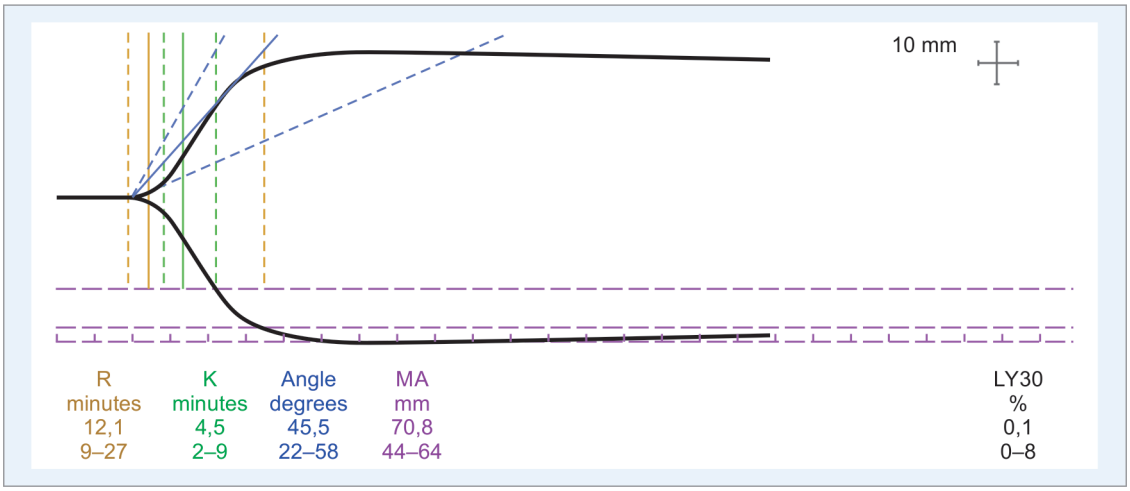


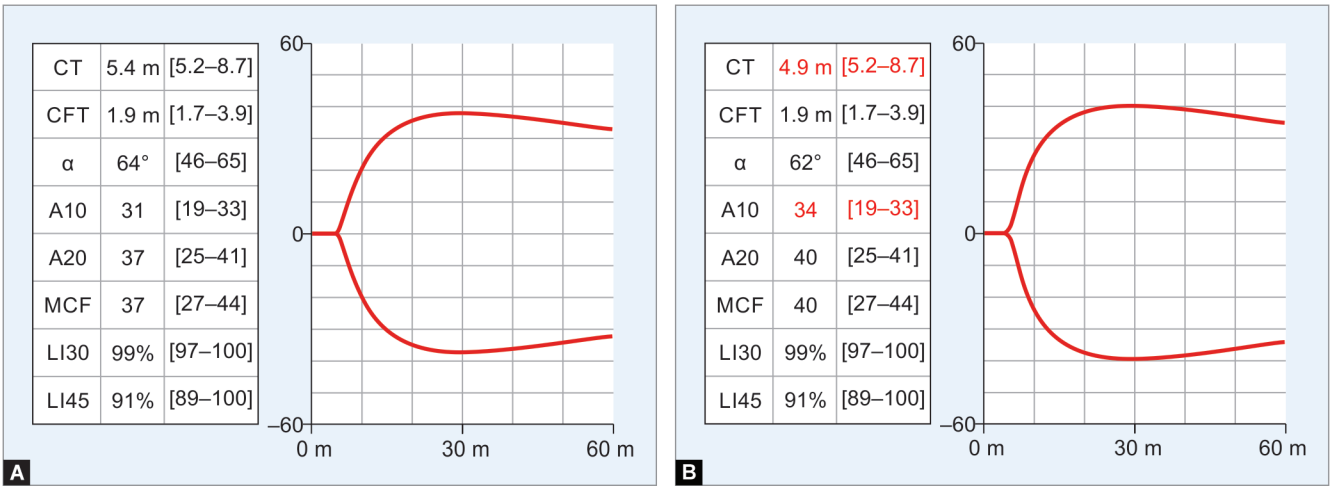
Fig. 3: The TEG tracing, obtained after rFVIII administration depicts shorter *R*- and *K*-time and a steeper α-angle

improvement of clot initiation and amplification (shorter *R*- and *K*-time and a steeper α-angle). During the postoperative period, TEG analysis together with conventional tests helps physicians understand patient hemostasis and tailor prophylactic rFVIII infusions.

Regling et al.[40] studied 160 pediatric patients aged from 2 weeks to 18 years who were evaluated for bleeding abnormalities. Seventy-eight were diagnosed with von Willebrand (VW) disease. In this subgroup, TEG parameters, such as *K*-time and maximum thrombus generation (MRTG) rate, were abnormal. Prolonged *K*-time and low MRTG were sensitive in detecting patients with VW factor activity below 30 IU/dL. The authors found the TEG test to be useful because it helped physicians in prompt identification of patients at risk of major bleeding. Furthermore, TEG assay also appeared to be beneficial in assessing the need for replacement therapy in minor acute bleeding.[40]

Rare coagulation diseases include deficiencies of coagulation factors such as fibrinogen, prothrombin, factor (F) V, FVII, FX, FXI, and FXIII, and the combined deficiency of FV + FVIII and vitamin K-dependent factors. In these patients, the clotting level activity does not always correlate with the risk of bleeding. Global coagulation tests, although not yet standardized, are believed to add information on the prediction of individual phenotypes and therefore aid the clinical management of affected patients.[41,42]

An inborn premature neonate was admitted to our NICU at 31-week gestation with a prenatally diagnosed ventriculomegaly. During follow-up, serial cranial ultrasound scans showed worsening posthemorrhagic ventricular dilatation. Due to the cerebral bleeding, standard coagulation tests were performed, and, on all occasions, a prolonged PT was noted. In addition to conventional tests, VCM™ was also used to better understand the neonate's global hemostasis (Fig. 4). Viscoelastic coagulation monitor tests consistently showed normal clot formation and lysis. However, vitamin K-dependent clotting factors II, VII, IX, and X were found to be deficient but the levels of these factors were not low enough to suggest an increased risk of spontaneous bleeding, as always demonstrated by VCM tests. Intravenous administration of vitamin K partially corrected the levels of these factors. The patient underwent surgery twice, once for ventricular reservoir placement and once for placement of a ventriculoperitoneal shunt. No bleeding complications were reported. Before surgery, the neonate was prophylactically treated with prothrombin complex concentrates and monitored for further administration with standard coagulation tests and VCM analysis. Viscoelastic traces



Figs 4A and B: The VCM trace of a premature neonate with a deficiency of vitamin K-dependent clotting factors. α, α-angle; A10, clot amplitude at 10 minutes; A20, clot amplitude at 20 minutes; CT, clotting time; CFT, clot formation time; LI30, lysis index at 30 minutes; LI45, lysis index at 45 minutes; MCF, maximum clot firmness; VCM tests showed a normal clot formation and lysis before (Fig. 4A) and after (Fig. 4B) administration of prothrombin complex concentrate

consistently demonstrated good clot formation and, together with standard coagulation tests, guided physicians in therapeutic decision making (Fig. 4) after surgery and for administration of prothrombin complex concentrate.

Conclusion

Viscoelastic coagulation tests, providing a rapid global assessment of hemostasis, appear to be useful to determine the neonatal coagulation profile, to identify neonatal patients at risk of developing postoperative bleeding and coagulation abnormalities, and therefore to guide blood product transfusions. Many neonatal units worldwide have incorporated viscoelastic testing into their practice and have been evaluating these tools in neonatology.

The promising results of preliminary studies on neonates have shown that viscoelastic tests may help overcome the limitations of conventional coagulation tests; there are advantages such as the feasibility of bedside testing, the availability of a panoramic view of the entire hemostatic process, and the need for minimal blood volumes. However, further testing is needed to support or refute the routine use of viscoelastic testing to guide hemostatic treatment compared to usual care in bleeding newborns. There is a need for larger, high-quality studies.

References

1. Wells M, Raja M, Rahman S. Point-of-care viscoelastic testing. BJA Educ 2022;22(11):416–423. DOI: 10.1016/j.bjae.2022.07.003.
2. Tripodi A, Chantarangkul V, Mannucci PM. Acquired coagulation disorders: Revisited using global coagulation/anticoagulation testing. Br J Haematol 2009;147(1):77–82. DOI: 10.1111/j.1365-2141.2009.07833.x.
3. Whiting D, DiNardo JA. TEG and ROTEM: Technology and clinical applications. Am J Hematol 2014;89(2):228–232. DOI: 10.1002/ajh.23599.
4. Andrew M. The relevance of developmental hemostasis to hemorrhagic disorders of newborns. Semin Perinatol 1997;21(1):70–85. DOI: 10.1016/s0146-0005(97)80022-5.
5. Monagle P, Massicotte P. Developmental haemostasis: Secondary haemostasis. Semin Fetal Neonatal Med 2011;16(6):294–300. DOI: 10.1016/j.siny.2011.07.007.
6. Andrew M, Paes B, Milner R, et al. Development of the human coagulation system in the full-term infant. Blood 1987;70(1):165–172. PMID: 3593964.
7. Andrew M, Paes B, Milner R, et al. Development of the human coagulation system in the healthy premature infant. Blood 1988;72(5):1651–1657. PMID: 3179444.
8. Christensen RD, Baer VL, Lambert DK, et al. Reference intervals for common coagulation tests of preterm infants (CME). Transfusion 2014;54(3):627–632. DOI: 10.1111/trf.12322.
9. Meybohm P, Zacharowski K, Weber CF. Point-of-care coagulation management in intensive care medicine. Crit Care 2013;17(2):218. DOI: 10.1186/cc12527.
10. Puetz J, Darling G, McCormick KA, et al. Fresh frozen plasma and recombinant factor VIIa use in neonates. J Pediatr Hematol Oncol 2009;31(12):901–906. DOI: 10.1097/MPH.0b013e3181c29c25.
11. Motta M, Del Vecchio A, Perrone B, et al. Fresh frozen plasma use in the NICU: A prospective, observational, multicentred study. Arch Dis Child Fetal Neonatal Ed 2014;99(4):F303–F308. DOI: 10.1136/archdischild-2013-304747.
12. Venkatesh V, Khan R, Curley A, et al. How we decide when a neonate needs a transfusion. Br J Haematol 2013;160(4):421–433. DOI: 10.1111/bjh.12095.
13. Carroll PD, Christensen RD. New and underutilized uses of umbilical cord blood in neonatal care. Matern Health Neonatol Perinatol 2015;1:16. DOI: 10.1186/s40748-015-0017-2.
14. Radicioni M, Mezzetti D, Del Vecchio A, et al. Thromboelastography: Might work in neonatology too? J Matern Fetal Neonatal Med 2012;(Suppl. 4):18–21. DOI: 10.3109/14767058.2012.714996.
15. Radicioni M, Bruni A, Bini V, et al. Thromboelastographic profiles of the premature infants with and without intracranial hemorrhage at birth: A pilot study. J Matern Fetal Neonatal Med 2015;28(15):1779–1783. DOI: 10.3109/14767058.2014.968773.
16. Amelio GS, Raffaeli G, Amodeo I, et al. Hemostatic evaluation with viscoelastic coagulation monitor: A Nicu experience. Front Pediatr 2022;10:910646. DOI: 10.3389/fped.2022.910646.
17. Panigada M, Meli A, Scotti E, et al. Viscoelastic coagulation monitor as a novel device to assess coagulation at the bedside. A single-center experience during the COVID-19 pandemic. Asaio J 2021;67(3):254–262. DOI: 10.1097/mat.0000000000001380.
18. Radicioni M, Massetti V, Bini V, et al. Impact of blood sampling technique on reproducibility of viscoelastic coagulation monitor (VCM™) system test results in the neonate. J Matern Fetal Neonatal Med 2022;35(25):6998–7004. DOI: 10.1080/14767058.2021.1933935.

19. Ghirardello S, Raffaeli G, Scalambrino E, et al. The intra-assay reproducibility of thromboelastography in very low birth weight infants. Early Hum Dev 2018;127:48–52. DOI: 10.1016/j.earlhumdev.2018.10.004.
20. Edwards R, Naik–Mathuria B, Gay A, et al. Parameters of thromboelastography in healthy newborns. Am J Clin Pathol 2008;130(1):99–102. DOI: 10.1309/LABNMY41RUD099J2.
21. Motta M, Guaragni B, Pezzotti E, et al. Reference intervals of citrated-native whole blood thromboelastography in premature neonates. Early Hum Dev 2017;115:60–63. DOI: 10.1016/j.earlhumdev.2017.09.014.
22. Sewell E, Forman KR, Wong ECC, et al. Thromboelastography in term neonates: An alternative approach to evaluating coagulopathy. Arch Dis Child Fetal Neonatal Ed 2017;102(1):F79–F84. DOI: 10.1136/archdischild-2016-310545.
23. Sokou R, Foudoulaki–Paparizos L, Lytras T, et al. Reference ranges of thromboelastometry in healthy full-term and pre-term neonates. Clin Chem Lab Med 2017;55(10):1592–1597. DOI: 10.1515/cclm-2016-0931.
24. Sokou R, Konstantinidi A, Stefanaki C, et al. Thromboelastometry: Studying hemostatic profile in small for gestational age neonates—A pilot observational study. Eur J Nucl Med Mol Imaging 2019;178(4):551–557. DOI: 10.1007/s00431-019-03331-w.
25. Forman KR, Wong E, Gallagher M, et al. Effect of temperature on thromboelastography and implications for clinical use in newborns undergoing therapeutic hypothermia. Pediatr Res 2014;75(5):663–669. DOI: 10.1038/pr.2014.19.
26. Stammer AH, Willet L, Fristoe L, et al. Coagulation monitoring during extracorporeal membrane oxygenation: The role of thromboelastogram. J Extra Corp Technol 1995;27(3):137–145. PMID: 10155358.
27. Phillips RC, Shahi N, Leopold D, et al. Thromboelastography-guided management of coagulopathy in neonates with congenital diaphragmatic hernia supported by extracorporeal membrane oxygenation. Pediatr Surg Int 2020;36(9):1027–1033. DOI: 10.1007/s00383-020-04694-0.
28. Peterson JA, Maroney SA, Zwifelhofer W, et al. Heparin–protamine balance after neonatal cardiopulmonary bypass surgery. J Thromb Haemost 2018;16(10):1973–1983. DOI: 10.1111/jth.14245.
29. Sokou R, Giallouros G, Konstantinidi A, et al. Thromboelastometry for diagnosis of neonatal sepsis-associated coagulopathy: An observational study. Eur J Pediatr 2018;177(3):355–362. DOI: 10.1007/s00431-017-3072-z.
30. Sokou R, Piovani D, Konstantinidi A, et al. A risk score for predicting the incidence of hemorrhage in critically ill neonates: Development and validation study. Thromb Haemost 2021;121(2):131–139. DOI: 10.1055/s-0040-1715832.
31. Venkatesh V, Curley A, Khan R, et al. A novel approach to standardised recording of bleeding in a high risk neonatal population. Arch Dis Child Fetal Neonatal Ed 2013;98(3):F260–F263. DOI: 10.1136/archdischild-2012-302443.
32. Raffaeli G, Pesenti N, Cavallaro G, et al. Optimizing fresh-frozen plasma transfusion in surgical neonates through thromboelastography: A quality improvement study. Eur J Pediatr 2022;181(5):2173–2182. DOI: 10.1007/s00431-022-04427-6.
33. Nogami K. The utility of thromboelastography in inherited and acquired bleeding disorders. Br J Haematol 2016;174(4):503–514. DOI: 10.1111/bjh.14148.
34. Sorensen B, Ingerslev J. Whole blood clot formation phenotypes in hemophilia A and rare coagulation disorders. Patterns of response to recombinant factor VIIa. J Thromb Haemost 2004;2(1):102–110. DOI: 10.1111/j.1538-7836.2004.00528.x.
35. Al Hawaj MA, Martin EJ, Venitz J, et al. Monitoring rFVIII prophylaxis dosing using global haemostasis assays. Haemophilia 2013;19(3):409–414. DOI: 10.1111/hae.12110.
36. Aghighi S, Riddell A, Lee C, et al. Global coagulation assays in hemophilia A: A comparison to conventional assays. Res Pract Thromb Haemost 2020;4(2):298–308. DOI: 10.1002/rth2.12295.
37. Makris M, Oldenburg J, Mauser–Bunschoten EP, et al. The definition, diagnosis and management of mild hemophilia A: Communication from the SSC of the ISTH. J Thromb Haemost 2018;16(12):2530–2533. DOI: 10.1111/jth.14315.
38. Bolton–Maggs PH, Pasi KJ. Haemophilias A and B. Lancet 2003;361(9371):1801–1809. DOI: 10.1016/S0140-6736(03)13405-8.
39. Ramiz S, Hartmann J, Young G, et al. Clinical utility of viscoelastic testing (TEG and ROTEM analyzers) in the management of old and new therapies for hemophilia. Am J Hematol 2019;94(2):249–256. DOI: 10.1002/ajh.25319.
40. Regling K, Kakulavarapu S, Thomas R, et al. Utility of thromboelastography for the diagnosis of von Willebrand disease. Pediatr Blood Cancer 2019;66(7):e27714. DOI: 10.1002/pbc.27714.
41. Peyvandi F, Palla R, Menegatti M, et al. Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: Results from the European Network of Rare Bleeding Disorders, J Thromb Haemost 2012;10(4):615–621. DOI: 10.1111/j.1538-7836.2012.04653.x.
42. Meneghetti M, Palla R. Clinical and laboratory diagnosis of rare coagulation disorders (RCDs). Thromb Res 2020;196:603–608. DOI: 10.1016/j.thromres.2019.09.006.

REVIEW ARTICLE

Holoprosencephaly

Akhil Maheshwari[1,2], Thierry AGM Huisman[3]

Received on: 15 December 2023; *Accepted on*: 10 January 2024; *Published on*: 26 March 2024

Abstract

Holoprosencephaly (HPE) is a complex malformation of the forebrain resulting from failed or incomplete division of the forebrain during the embryonic period. It is the most frequently seen developmental abnormality of the forebrain in humans; the incidence is nearly 1 of every 250 human embryos. However, most of these embryos do not survive and are lost to miscarriage. At birth, the prevalence is 1 in 8,000–10,000 live births and stillbirths. In HPE, the cleavage of the forebrain (prosencephalon) into the right and left hemispheres, deep brain structures, and the olfactory and optic bulbs and tracts remains incomplete. These central nervous system (CNS) defects develop during the first few 2–3 weeks of the fetal period. The etiopathogenesis is unclear, although both syndromic and isolated HPE can be heritable. The condition involves multiple systems, including the central nervous system, eyes, hearing, olfactory, gastrointestinal system, and genital tracts can be most severely affected. No specific treatment is known. Careful clinical and genetic evaluation is necessary for symptomatic management and for counseling families. In this article, we present our own clinical and imaging experience and combine it with an extensive search in the databases PubMed, EMBASE, and Scopus. To avoid bias, keywords were identified from discussions in our own group and from PubMed's Medical Subject Heading (MeSH) thesaurus.

Keywords: 7-dehydrocholesterol reductase, Alobar, Ambiguous genitalia, Anophthalmia, Arhinencephaly, Asegmentation, pseudotrisomy 13; genoa syndrome (semilobal hpe); and brachial amelia, Azygos anterior cerebral artery, Butterfly sign, Caudal dysgenesis, Cavum septi pellucidi, Ccr4-not transcription complex, Cell adhesion associated, Cell adhesion molecule-related/downregulated by oncogenes, Cerebellar hypoplasia, Chiari I malformation, Chromosomal errors, Circle of Willis, Cleft lip, Cripto, Culler–Jones syndrome, de Morsier syndrome, Delta-like canonical notch ligand 1, Demyer, Dispatched RND transporter family member, Dispatched RND transporter family member 1, *EGF-CFC* family member, Fibroblast growth factor 8, Fibroblast growth factor 8 (*FGF8*), Fibroblast growth factor receptor 1, Fibroblast growth factor receptor 1 (*FGFR1*), Forebrain, Forkhead *BOX H1 FOXH1*, Forkhead box protein h1, Frenulum, Frontonasal dysplasia, GLI family zinc finger 2, Growth arrest specific 1, Growth arrest specific 1 (*GAS1*), Hartsfield syndrome, Holoventricle, Hypothalamic hamartoblastomata, Infant, Lysine methyltransferase 2d, Lysine methyltransferase 2d (*KMT*2d), Malformation, Meckel syndrome, Median cleft face syndrome, Microform, Microphthalmia, Microtia-anotia, Middle interhemispheric fusion variant, Midline cleft syndrome, Monoventricle, Neonate, Newborn, Nodal growth differentiation factor, Nodal growth differentiation factor (*NODAL*), Olfactory bulb, Oncogene regulated, Optic bulb, Pallister–Hall syndrome, Patched 1, Patched 1 (*PTCH1*), Pathogenic copy number variations, Polydactyly, Prokineticin receptor, Prosencephalon, Protein patched homolog, Protein patched homolog 1 (*PTCH1*), Protein phosphatase 1 regulatory subunit 12a, Protein phosphatase 1 regulatory subunit 12a (*PPP1R*12a), Rad21 cohesin complex component, Rubinstein–Taybi syndrome, Semilobar, Septo-optic dysplasia (SOD), Septo-preoptic, SHH mutations, Sine oculis homeobox homolog, Sine oculis homeobox homolog 3, Single maxillary central incisor, Smith-Lemli-Opitz, Smith-Lemli-Opitz syndrome, Snake under the skull, Solitary median maxillary central incisor, Sonic hedgehog, Stag2 cohesin complex component, STIL centriolar assembly protein, Structural maintenance of chromosomes 1a, Structural maintenance of chromosomes 3, Subunit 1, Sufu negative regulator of hedgehog signaling, Sylvian fissures, Syntelencephaly, TGF-beta-induced factor homeobox factor 1, Trisomy 13, Yim–Ebbin syndrome, *ZIC2* mutations, Zinc finger protein, Zinc finger protein *ZIC2*.

Newborn (2024): 10.5005/jp-journals-11002-0083

Key Points

- Holoprosencephaly (HPE) is characterized by a failure to define the midline of the forebrain and midface.
- There are different degrees of severity ranging from a completely alobar HPE to milder forms with some regional or localized segmentation of the two lobes.
- The three most severe, best-known categories of HPE are alobar, semilobar, and lobar, but there may also be several minor variations such as the septopreoptic type, microform, solitary median maxillary central incisor, and frontonasal dysplasia.
- The etiopathogenesis remains unclear, although a large number of associated chromosomal and genetic abnormalities have been identified.
- The condition affects multiple systems, including the central nervous system, eyes, hearing, olfactory, the gastrointestinal system, and the genital tracts.
- No specific treatment is known. Careful clinical and genetic evaluation is necessary for symptomatic management and for counseling families.

[1]Pediatrics, Louisiana State University-Shreveport, Shreveport, Louisiana, United States of America

[2]Global Newborn Society, Clarksville, Maryland, United States of America

[3]Department of Radiology, Texas Children's Hospital, Houston, Texas, United States of America

Corresponding Authors: Akhil Maheshwari, Pediatrics, Louisiana State University-Shreveport, Shreveport, Louisiana; Global Newborn Society, Clarksville, Maryland, United States of America; Phone: +7089108729, e-mail: Akhil@globalnewbornsociety.org; Thierry AGM Huisman, Department of Radiology, Texas Children's Hospital, Houston, Texas, United States of America; e-mail: huisman@texaschildrens.org

How to cite this article: Maheshwari A, Huisman TAGM. Holoprosencephaly. Newborn 2024;3(1):45–60.

Source of support: Nil

Conflict of interest: Dr Akhil Maheshwari and Dr Thierry AGM Huisman are associated as the Editorial board members of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of these Editorial board members and their research group.

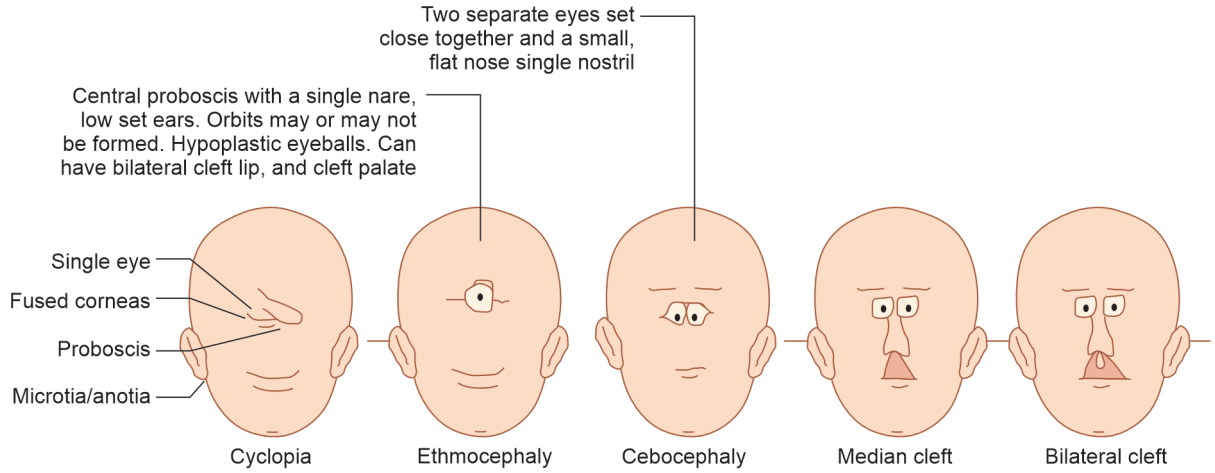


Fig. 1: The holoprosencephaly spectrum shows a range of dysmorphic features that indicate the severity of the disability

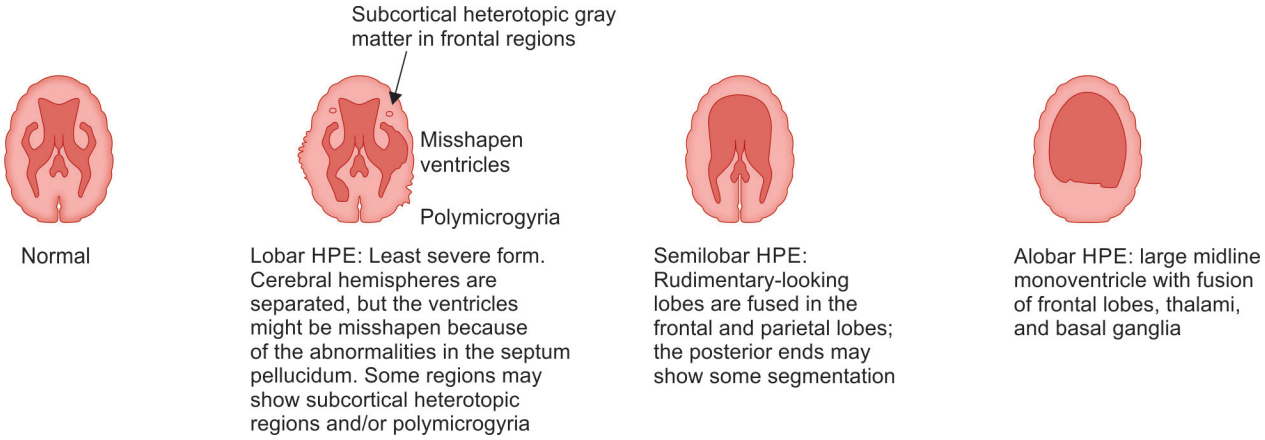


Fig. 2: These figures show a basic classification of altered brain structure in holoprosencephaly. However, advances in neuroimaging and the clinical awareness have helped identify many more subtypes of the condition

Introduction

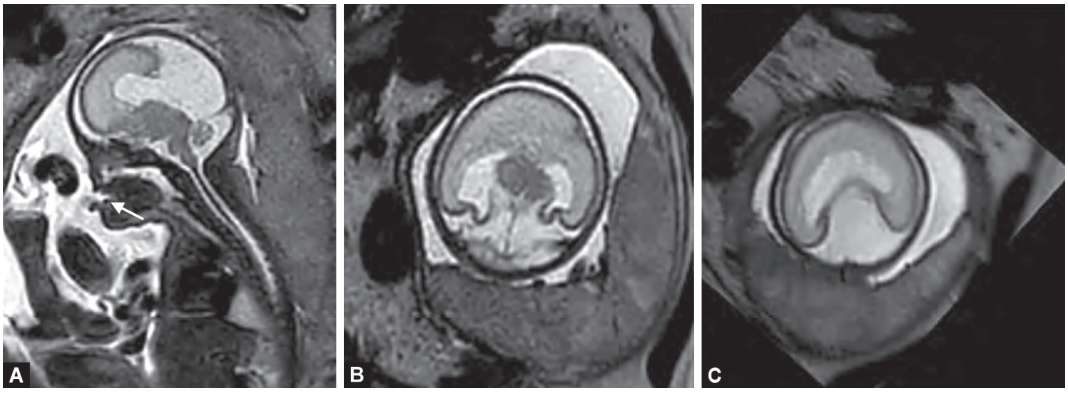
Holoprosencephaly (HPE) is a complex malformation of the brain resulting from an incomplete midline cleavage of the forebrain (prosencephalon).[1–3] It includes a wide spectrum of intracranial and craniofacial midline defects and a myriad of clinical manifestations, consisting of neurologic impairment and dysmorphism of the brain and face.[4] It is the most common malformation of forebrain development and is seen both sporadically or in syndromic associations.[2,4] The defect associated with HPE occurs at approximately 2–3 weeks post-conception and is a disorder of gastrulation.[5,6] The anomaly has been described using several synonyms, including HPE; midline cleft syndrome; DeMyer sequence; isolated, non-syndromic, or non-chromosomic HPE; familial HPE, arr hinencephaly, or cyclopia.[1,4,7–11]

The etiopathogenesis of HPE still remains unclear. We know that the forebrain neuroectoderm (FNP) plays a critical role in the growth and development of the neural structures in the facial midline.[12] In terms of structure, the FNP serves as a scaffold, and in terms of neurological development, its proximity to the developing forebrain allows for molecular signals to reach these developing neural crest-derived cranial mesenchyme structures.[13] Loss of neural crest-derived mesenchyme in the mid-facial region interrupts the growth of these midline structures.[14] However, further in-depth understanding is still missing. In this article, we present our own clinical and imaging experience and combine it with an extensive search in the databases PubMed, EMBASE, and Scopus. To avoid bias, keywords were identified from discussions in our own group and from PubMed's Medical Subject Heading (MeSH) thesaurus.

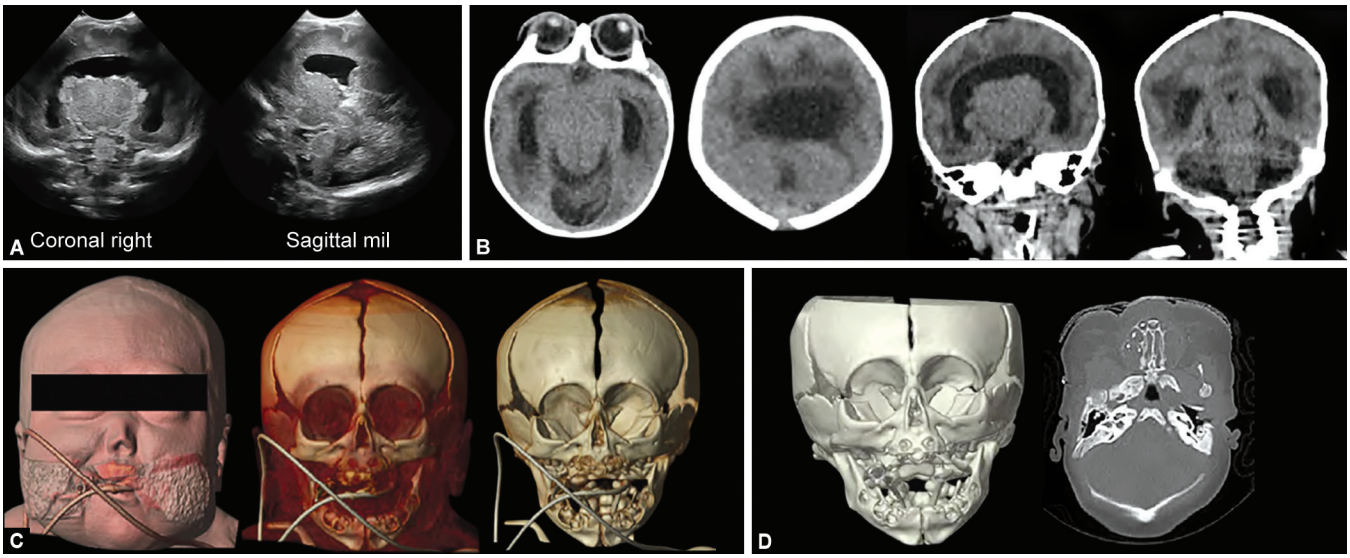
Classification

There are several different types of holoprosencephaly.[4] Mild manifestations may include the presence of a single central incisor (one upper front tooth rather than the usual two), whereas the severe end of the spectrum shows as a single central eye (cyclopia) and related facial abnormalities (Fig. 1).[10] Intermediate manifestations often include a cleft lip. The continuum of craniofacial anomalies is seen in nearly 80% of individuals with HPE.[1]

Holoprosencephaly is usually identified in the following categories. A basic classification of altered brain structure is shown in Figure 2. However, the following section provides details on the increasing information on various subtypes of HPE:

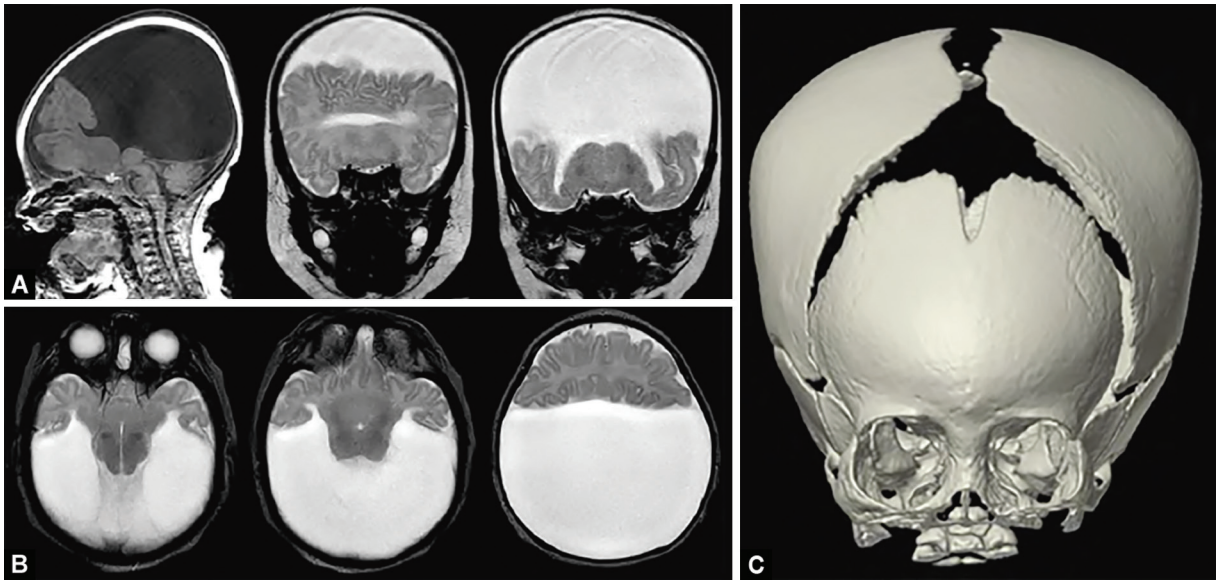


Figs 3A to C: Sagittal and axial T2-weighted fetal MRI of a fetus with alobar holoprosencephaly. A large monoventricle was noted. The thalami were fused in the midline and no interhemispheric fissure was noted. There was a hypoplastic nose and bilateral cleft lip and palate (arrow). The tongue is outlined by T2-hyperintense amniotic fluid which extended towards the base of the skull. No hard or soft plate was seen separating the oral cavity from the nasal cavity. In this infant, a clubfoot was also noted

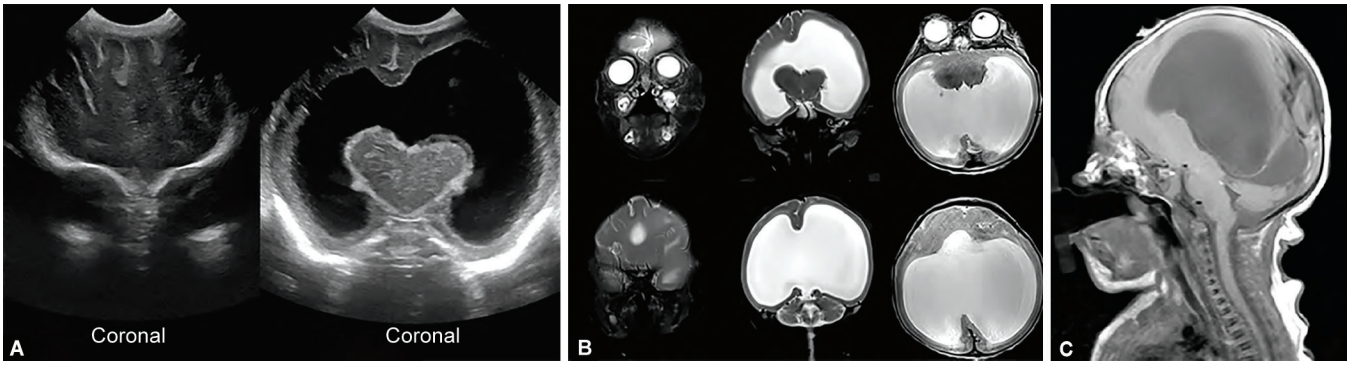


Figs 4A to D: (A) Coronal and sagittal ultrasound of a 1-day-old female infant with alobar holoprosencephaly showed a large cerebrospinal fluid-filled monoventricle extending across the midline. In addition, the thalami were fused. The choroid plexus can be seen covering the fused, midline gray matter. In the sagittal view, the junction between the mesencephalon and diencephalon appeared dysmorphic, but the cerebellum looked nearly normal; (B) Axial and coronal CT of the same neonate confirmed the diagnoses of alobar holoprosencephaly. There was non-separation of the telencephalon with fused cerebral hemispheres and central gray matter structures, and bilateral pachygyria. In addition, hypotelorism is noted; (C) Multiple 3D reconstructions of the CT dataset of the same neonate showed coexistent maxillofacial anomalies including a single air-filled nostril and marked hypotelorism; (D) Follow-up 3D and axial CT of the same neonate showed better details of a single midline maxillary central incisor, choanal atresia, a diffusely opacified and obstructed single nasal cavity, and relative medialization of the bilateral inferior orbital fissures/foramen

- Alobar HPE: This is the most severe form of HPE (Figs 2 to 5).[15] There is an enlarged midline mono ventricle (holo ventricle) with a fusion of the frontal lobes and the midline gray matter structures such as the thalami and basal ganglia.[16] The corpus callosum and the third ventricle are typically absent. The facial features may include a single eye-like structure, cyclopia, in a fused single orbit.[17] Many infants have a single overriding nose-like structure, the proboscis.[18,19] In some, the eyes might be closely-spaced with a short inter-orbital distance, which has been described as ethmocephaly.[20] Cebocephaly is a variant with closely spaced eyes separated by a single-nostril nose.[21] Another group of patients may have anophthalmia or microphthalmia.[22] Milder ends of the spectrum may show premaxillary agenesis with median cleft lip, closely spaced eyes, depressed nasal ridge, bilateral cleft lip, or normal-looking facial appearance.[10]
- Semilobar HPE (partial segmentation): Rudimentary-looking left and right lobes are fused in the frontal and parietal lobes; the posterior ends may show some segmentation.[23] The corpus callosum may be hypoplastic.[24] Varying non-separation of deep gray nuclei. The eyes are closely spaced and some patients may even show anophthalmia/microphthalmia.[25] The nasal ridge might be depressed or absent. There might be midline cleft lip/palate. Others may show bilateral cleft lip with median process representing the philtrum-premaxilla anlage (Figs 6 to 8).[10,26]

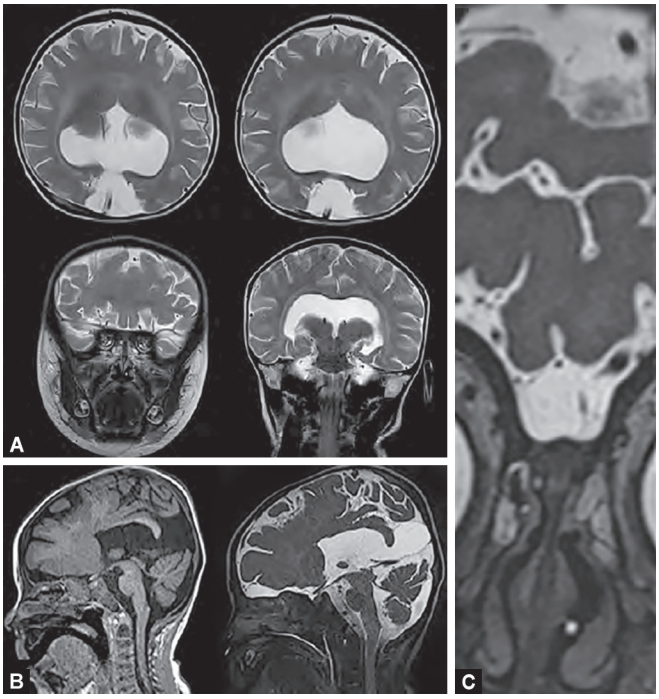


Figs 5A to C: (A) Sagittal T1-, coronal, and axial T2-weighted MRI of a 25-day-old female infant with alobar holoprosencephaly showed a large CSF filled monoventricle that connected with prominent temporal horns. The massively-dilated monoventricle extended posteriorly with bilateral high-grade thinning of the parieto-occipital lobe; (B) The fused central gray matter and frontal lobes can be seen in coronal and axial T2-weighted images. The falx cerebri, corpus callosum and olfactory bulbs were absent; (C) 3D surface-shaded CT reconstruction of the skull showed the lack of a metopic suture and marked hypotelorism



Figs 6A to C: (A) Coronal prenatal US in a 1-day-old male neonate with alobar holoprosencephaly. Imaging showed fusion of both frontal lobes and the central gray matter with a monoventricle; (B) Coronal and axial T2-weighted MRI of the same neonate confirmed the ultrasound findings of fusion of the frontal lobes and central gray matter across the midline, and a monoventricle. The posterior cerebral hemispheres were separated by a hypoplastic falx cerebri, confirming the diagnosis of semilobar holoprosencephaly. The cortical gray matter of the parietal and occipital lobes was compressed and thinned due to the high-grade ventriculomegaly. Moderate hypotelorism and a single azygos artery were noted on the coronal MR image; (C) Sagittal T1-weighted MRI showed additional maxillofacial anomalies including a cleft palate. The large bilateral lip clefts in this scan are obscured by the pacifier

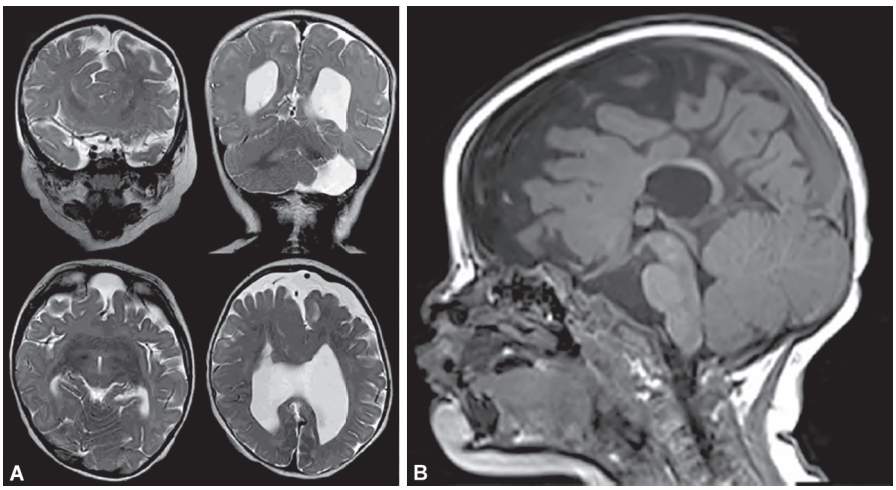
- Lobar HPE (the least severe form of a segmentation): The cerebral hemispheres are separated, although the ventricles might be misshapen because of the absence of the septum pellucidum (Fig. 8). There may also be some regions with polymicrogyria and/or subcortical heterotopias. Some patients have bilateral cleft lip, closely spaced eyes, depressed nasal ridge, and a relatively normal facial appearance (Fig. 9).[4,27]
- Middle interhemispheric fusion variant (MIHF/MIHV or syntelencephaly): In these infants, the posterior frontal and parietal lobes, the basal ganglia, and the thalami fail to separate.[28,29] The anterior ends of the frontal lobes and the occipital lobes are well-separated; the body of the corpus callosum may be absent but the genu and splenium are preserved.[29] These infants may also have heterotopic gray matter.[30] Clinically, these infants may show closely-spaced eyes and a depressed or narrow nasal bridge, but the overall facial appearance may not be obviously abnormal.[10]
- On imaging, these infants may show an azygos anterior cerebral artery, a dorsal cyst, cerebellar abnormalities such as a Chiari I malformation or cerebellar hypoplasia, and polymicrogyria.[31] The Sylvian fissures may be abnormally connected across the midline.[32] Some patients may show cortical dysplasia and subcortical heterotopic gray matter. The septum pellucidum may be hypoplastic (Fig. 10).[33]
- Septo-preoptic Type: Nonseparation is restricted to the septal and/or preoptic regions.[34]



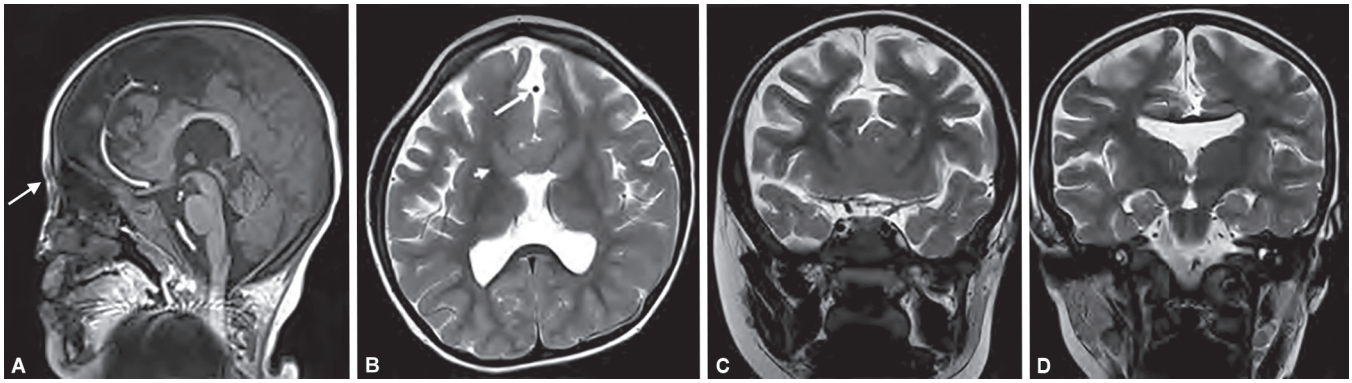
Figs 7A to C: (A) Axial and coronal T2-weighted MRI in young male child showed semilobar holoprosencephaly characterized by a monoventricle and a fusion of the anterior lobes across the midline. The parietal and occipital lobes were separated by a hypoplastic falx cerebri. The olfactory bulbs and olfactory sulci were absent. The hippocampi were small and malrotated; (B) Sagittal T1- and T2-weighted MRI of the same child show that the monoventricle communicated with a posterior cyst that displaced the vein of Galen inferiorly and the pineal gland posteriorly. The corpus callosum was malformed with a missing anterior and a dysplastic posterior part. The superior and inferior parts of the vermis were hypoplastic; the vermis and the torcula were elevated; (C) High-resolution T2-weighted coronal MRI of the skull base showed an "empty" anterior part without an olfactory sulcus, tract, or bulb

- Microform HPE: These infants show HPE-related craniofacial anomalies but now obvious structural brain defects on imaging.[1] The clinical spectrum may range from microcephaly; closely-spaced eyes, ophthalmologic anomalies such as refractive errors, ptosis, micro cornea and coloboma; sharp, narrow nasal bridge, nasal pyriform aperture stenosis, anosmia/hyposmia due to hypoplastic olfactory tracts and bulbs; midface retrusion; absent superior labial frenulum, bilateral cleft lips, and a single central maxillary incisor.[10,35–37] Many infants have delayed development.[10,36]
- Solitary median maxillary central incisor (SMMCI) is a rare dental anomaly (Fig. 11).[37,38] These patients show a single, midline central maxillary incisor, midpalatal vomerine ridge, and a v-shaped palate.[37] It is usually considered as a minor variant of HPE. There might be multiple, mainly midline defects of development resulting from unknown factor(s) operating *in utero* about the 35–38th day from conception. The etiology is uncertain, although a missense mutation in the *SHH* gene at 7q36 may be associated.[37] Most patients have associated choanal atresia, midnasal stenosis, and/or pyriform aperture stenosis.[39]
- Frontonasal dysplasia: It is also known as median cleft face syndrome, and is another rare disorder characterized by midline defects involving the face, head, and central nervous system (Figs 12 and 13).[40] It is considered by some to be a less severe manifestation of the HPE spectrum. These patients show hypertelorism, cleft lip/palate, and absence of the nasal tip.[41,42] Cranial imaging shows a cranium bifidum occultum (cleft skull), ethmoidal cephalocele, agenesis of the corpus callosum, and ventriculomegaly.[43,44]

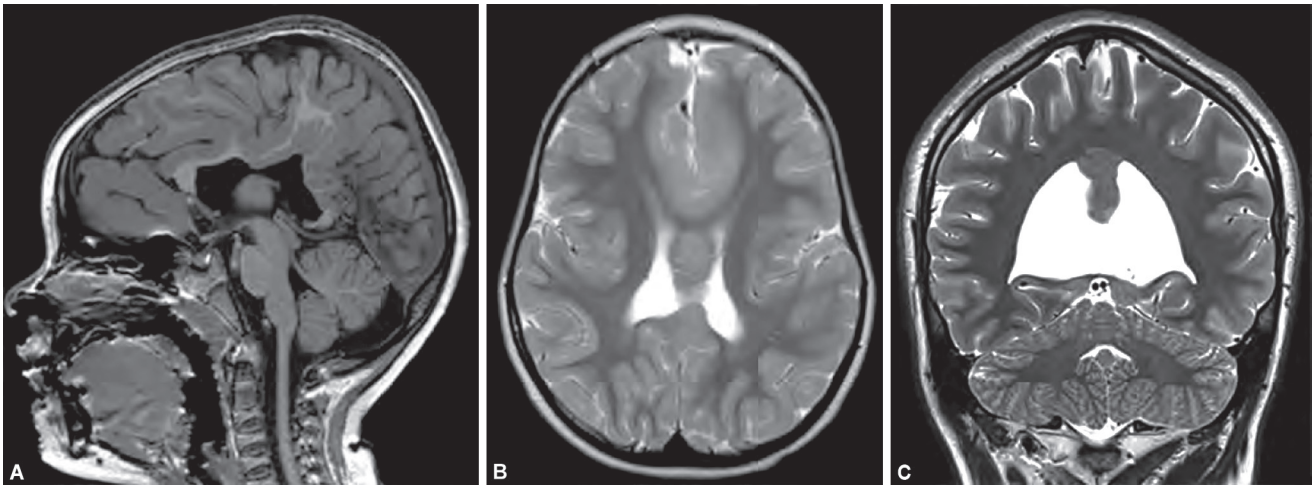
Patients with HPE may also have other structural CNS findings that are not specific to HPE, such as macrocephaly due to hydrocephalus, Dandy-Walker malformation, neuronal migration anomalies, abnormal circle of Willis, and caudal dysgenesis.[10] Seizures are seen in nearly half of all patients with HPE and are relatively easy to control with medical therapy in 50% of affected children.[1,4,5] The need for medication, not the occurrence of seizures, indicates a higher risk of having cortical dysplasia.[1,4,5]



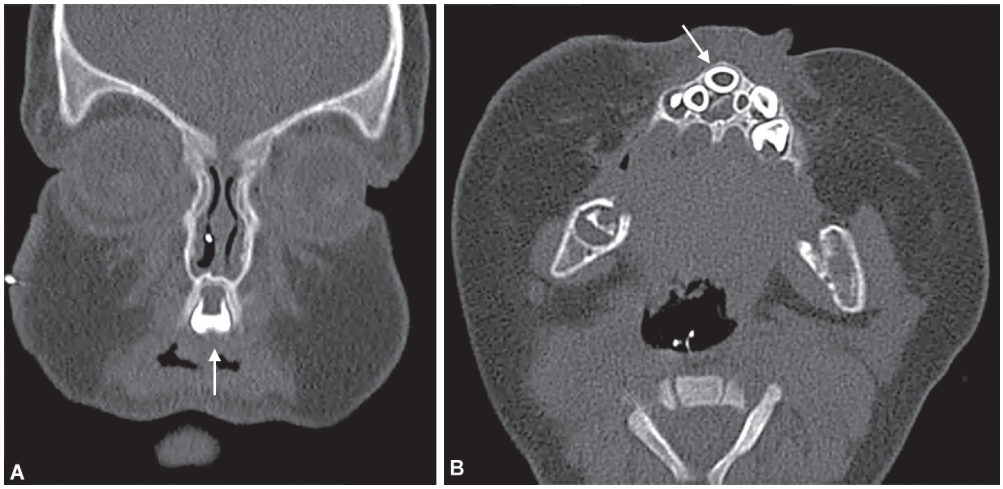
Figs 8A and B: (A) Coronal and axial T2-weighted MRI in a male infant with semilobar holoprosencephaly. The frontal lobes, anterior basal ganglia, and hypothalami were fused. The septum pellucidum, anterior falx, and the frontal horns of the lateral ventricles were missing. An unpaired, single azygos artery was noted. Heterotopic gray matter was seen anterior to the malformed hypoplastic corpus callosum. In addition, a cleft was seen within the left cerebellar hemisphere; (B) Sagittal T1-weighted MRI of the same patient confirmed the malformed corpus callosum. The rostrum, genu, and the anterior trunk of the corpus callosum were lacking; the posterior corpus callosum including splenium was visible



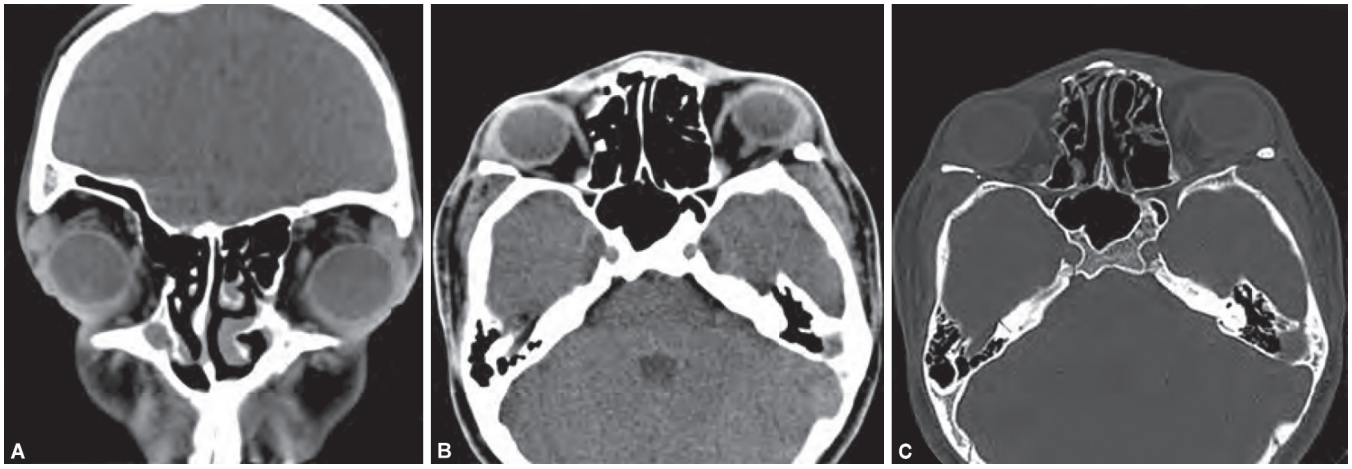
Figs 9A to D: Sagittal T1-, axial, and coronal T2-weighted MRI of a female child with lobar holoprosencephaly. There was fusion of the cortical gray matter across the midline of the inferior posterior frontal lobes, although the interhemispheric fissure and the falx were nearly completely developed. The septum pellucidum was absent. The ventricles were mildly dysmorphic. An azygous cerebral artery was noted (arrow), the anterior basal ganglia were dysmorphic, and the anterior corpus callosum was missing. The posterior part of the corpus callosum was developed. The nose and nasal bridge were severely hypoplastic (arrow)



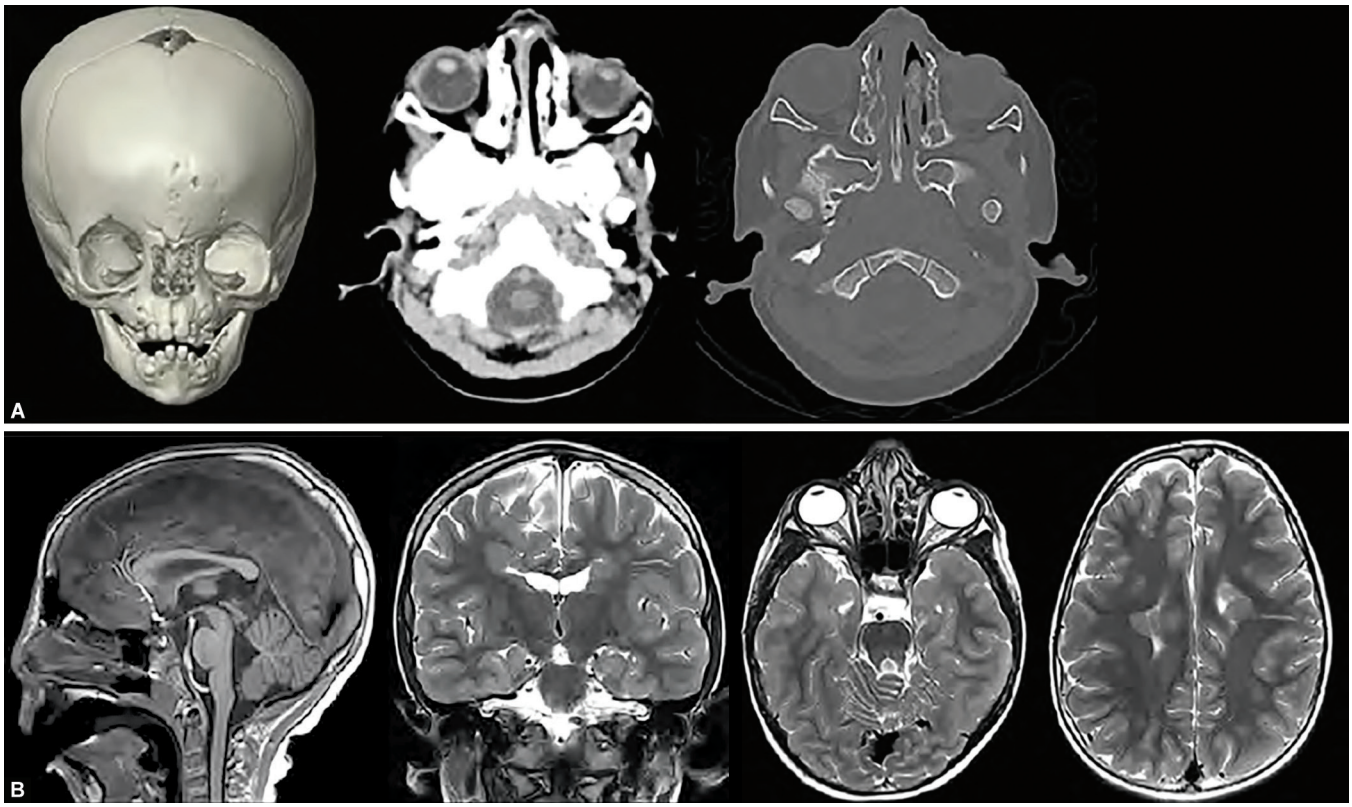
Figs 10A to C: Sagittal T1-, axial, and coronal T2-weighted MRI in a young female child show classical syntelencephaly with midline fusion of the cerebral hemispheres. The anterior and posterior parts of the corpus callosum were intact



Figs 11A and B: Coronal and axial CT of the face of an infant in bone algorithm. There was an isolated central incisor (arrows) in the alveolar ridge of the maxilla. Additional findings were stenosis of the pyriform aperture and a bony ridge along the undersurface of the hard palate. There were no intracranial brain anomalies



Figs 12A to C: Coronal and axial CT (soft tissue and bone algorithm) of the face in a child with nasal dysplasia. Prominent hypertelorism was seen in combination with frontal nasal dysplasia. There was a cleft palate at birth that had already been repaired. The brain was unremarkable



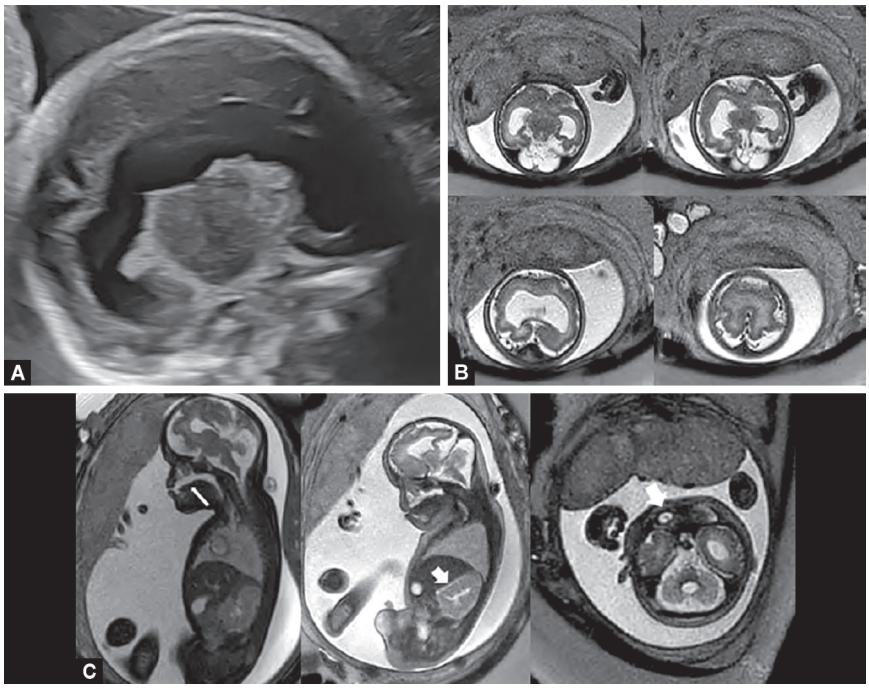
Figs 13A and B: (A) 3D, axial soft tissue, and bone algorithm CT of the face in a 1-month-old male infant patient with frontonasal dysplasia showed a hypoplastic nasal bridge and nose in conjunction with hypertelorism; (B) Sagittal T1-, coronal, and axial T2-weighted MRI of the same patient showed heterotopic gray matter along the lateral ventricles, a hypoplastic nose, absence of the left olfactory bulb, and hypertelorism

Etiology

Holoprosencephaly-related CNS defects begin to develop during the first few 2–3 weeks of the embryonic period.[25,45] It is the most frequently seen developmental abnormality of the forebrain in humans; the incidence is nearly 1 of every 250 human embryos.[1] Most embryos with HPE do not survive and are lost to miscarriage.[15] At birth, the prevalence is 1 in 8,000–10,000 live births and stillbirths.[5,46] Syndromic and isolated HPE can both be heritable.[10,47] Within the United States, certain ethnic groups, including African-American, Hispanic, and Pakistani communities, appear to exhibit slightly elevated prevalence rates.[4] This phenomenon is likely due to reduced rates of prenatal diagnosis and associated terminations within these groups.[5,48]

Chromosomal Abnormalities

About a third of all infants born with HPE have chromosomal abnormalities.[1,46] Chromosomal errors, including numeric



Figs 14A to C: (A) Coronal prenatal ultrasound study of a fetus with confirmed diagnosis of trisomy 13 showed a large single ventricle extending across the midline outlining the fused thalami that were partially covered by the choroid plexus. These findings are consistent with the diagnosis of holoprosencephaly; (B) Axial T2-weighted fetal MR images of the same fetus showed a large monoventricle with hemispheric white matter crossing the midline and fusion of the central gray matter. The posterior cerebral hemispheres were partially separated by a hypoplastic falx cerebri, which is compatible with the diagnosis of semilobar holoprosencephaly. There was also bilateral anomalous gyration and sulcation of cerebellar hemispheres; (C) Sagittal and axial T2-weighted fetal MRI of the same fetus show several additional findings, including bilateral cleft lips and cleft hard and soft palate (thin arrow). The nasal bridge was hypoplastic, flattened, and dysmorphic with absent nasal columella and nares. The fetus also showed midface hypoplasia with bilateral microphthalmia (short arrow). The kidneys were enlarged (short arrow)

abnormalities with an extra chromosome(s) or structural alterations with missing or added genetic material have been associated.[5] Trisomy 13 has been identified in up to 40–60% of all cases of HPE (Fig. 14); nearly 70% of these cases have arhinencephaly.[10,49,50]

Structural abnormalities of almost all chromosomes have been noted in HPE. Deletions or duplications involving various regions of 13q, and del (18p), del (7) (q36), dup (3) (p24-pter), del (2) (p21), and del (21) (q22.3) have been frequently reported.[10,51]

Single Gene Abnormalities

Syndromic HPE: About 18–25% of patients with a genetic cause have a recognizable syndrome.[10] The syndromic forms of HPE can be inherited in an autosomal recessive (AR) or autosomal dominant (AD) manner.[10,25] One example is the AR Smith-Lemli-Opitz syndrome (SLOS), where there are associated abnormalities in the face and the cerebral structure.[52] Smith-Lemli-Opitz syndrome is a variable genetic disorder marked by microcephaly, stunting, mild-to-moderate developmental delay, and other birth defects including facial dysmorphism, cleft palate, congenital heart defects, syndactyly involving 2nd and 3rd toes, polydactyly, and ambiguous genitalia.[53] About 10% of patients have altered cholesterol signaling related to the enzyme 7-dehydrocholesterol reductase.[54]

Genetic syndromes that are associated with HPE include Hartsfield syndrome (mutations in the fibroblast growth factor receptor 1 *FGFR1* gene; ectrodactyly, cleft lip/palate), agnathia-otocephaly complex (very small chin, ear anomalies), and Pallister-Hall syndrome (extra fingers/toes, hypothalamic hamartoblastomas, anal anomalies).[55–57] Holoprosencephaly is also seen in Cell adhesion molecule-related/down-regulated by oncogene *CDON1* gene has been identified in patients with congenital heart defects and abnormalities in the gall bladder, kidneys, and the radii.[58] Rubinstein-Taybi syndrome and Meckel syndrome have also been associated.[59,60] Kallman syndrome (Mutations in fibroblast growth factor receptor-1 *FGFR1* or *FGF8* identified in an AD form with incomplete penetrance may have some similarities with HPE.[61] Finally, mutations in prokineticin receptor-2 *PROKR2* and prokineticin-2 *PROK2 have been reported in* heterozygous, homozygous, and compound heterozygous states.[62,63]

Our understanding of the etiopathogenesis of syndromic HPE is patchy and remains limited to only a few conditions. In addition to Hartsfied syndrome, some information is now emerging in 13q deletion syndrome (ZIC 2), 18p deletion syndrome (TGIF1), Smith Lemli Opitz (SLO, DHCR7), Steinfeld (CDON or variants), Culler Jones (GLI2), hydrolethalus (HYLS1 and KIF7), and the Pallister-Hall (GLI3) syndrome.[47,57,64–68]

Non-syndromic HPE accounts for approximately 25–50% of all cases of HPE, affecting nearly 1 in 10,000 newborns.[10,46] Autosomal dominant -inherited mutations in 13 genes that are known to promote hemispheric fission have been identified most frequently. Most of these genes identified in HPE cluster around a few signaling pathways involving SHH, its receptor Protein patched homolog 1 PTCH1, *TGF-beta-induced factor homeobox factor 1* TGIF1 (a regulator of SHH), SIX3 (sine oculis homeobox homolog 3, a regulator of SHH),

and the Zinc finger protein ZIC2, which is needed for activation of nodal growth differentiation factor NODAL (upstream of SHH) and for ciliogenesis.[69–76] Other genes include forkhead box protein H1 (*FOXH1),* cell adhesion associated, oncogene regulated *(CDON),* fibroblast growth factor 8 *(FGF8),* and GLI family zinc finger 2 *(GLI2).*[77–80] The gene SHH promotes the eye field to separate into two distinct eyes.[81] The *SIX3* gene is needed for lenticular and retinal development.[82]

Patients with *SHH* mutations (5–6%) may be part of large kindreds, but many families may not be identified until severely affected probands are seen.[83,84] The *ZIC2* mutations are seen in about 5% of infants with bitemporal narrowing, upslanted palpebral fissures, large ears, short noses with anteverted nares, and a broad, deep philtrum.[85–87] Some may have renal abnormalities.[88] The *ZIC2* mutations are seen frequently in patients with the middle interhemispheric fusion variant.[89] The *SIX3* (<1%) and *TGIF* mutants (3%) may also be identified in severely afflicted probands.[74,90] The proportion of cases caused by new gene mutations is estimated to be approximately 70–80% for *ZIC2,* 10–30% for *SHH*, and 10–20% for *SIX3*.[84,86,88,90,91]

Even though many genes express signaling mediators that are plausibly involved in the pathophysiology of HPE as we understand it currently, mutations have been identified in only <2% of all patients who have this syndrome. These include Cell adhesion molecule-related/down-regulated by oncogenes *(CDON),* CCR4-NOT transcription complex, subunit 1 (*CNOT1),* dispatched RND transporter family member 1 (*DISP1*), delta like canonical notch ligand 1 (*DLL1*), fibroblast growth factor 8 (*FGF8),* FGF receptor 1 (*FGFR1),* lysine methyltransferase 2D (*KMT2D*), protein phosphatase 1 regulatory subunit 12A (*PPP1R12A*), RAD21 cohesin complex component (*RAD21*), structural maintenance of chromosomes 1A (*SMC1A*), structural maintenance of chromosomes 3 (*SMC3*), STAG2 cohesin complex component (*STAG2*), and STIL centriolar assembly protein (*STIL*).[55,92–100] Variants in several other mechanistically plausible candidate genes are also seen very infrequently; these include *FOXH1*, growth arrest specific 1 (*GAS1)*, nodal growth differentiation factor *(NODAL)*, patched 1 (*PTCH1),* negative regulator of hedgehog signaling *(SUFU)*, and the EGF-CFC family member *(CRIPTO,* formerly *TDGF1*).[77,101–105] Although pathogenic variants of GLI family zinc finger 2 (*GLI2)* were described as a cause of HPE, recent studies suggest this to not be the case. *GLI2* mutations seem to result in a phenotype characterized by pituitary anomalies, polydactyly, and subtle facial features that resemble Culler-Jones syndrome more than HPE.[106,107]

Associations with unknown genetic basis: Associations caudal dysgenesis; pseudotrisomy 13; genoa syndrome (semilobar HPE); and brachial amelia, cleft lip, and holoprosencephaly (Yim–Ebbin syndrome).[108–115]

Pathogenic Copy Number Variations (CNVs)

Chromosomal microarrays have identified pathogenic CNVs (including loci known to be associated with HPE) in 10% of all patients.[51,116]

Environmental Factors

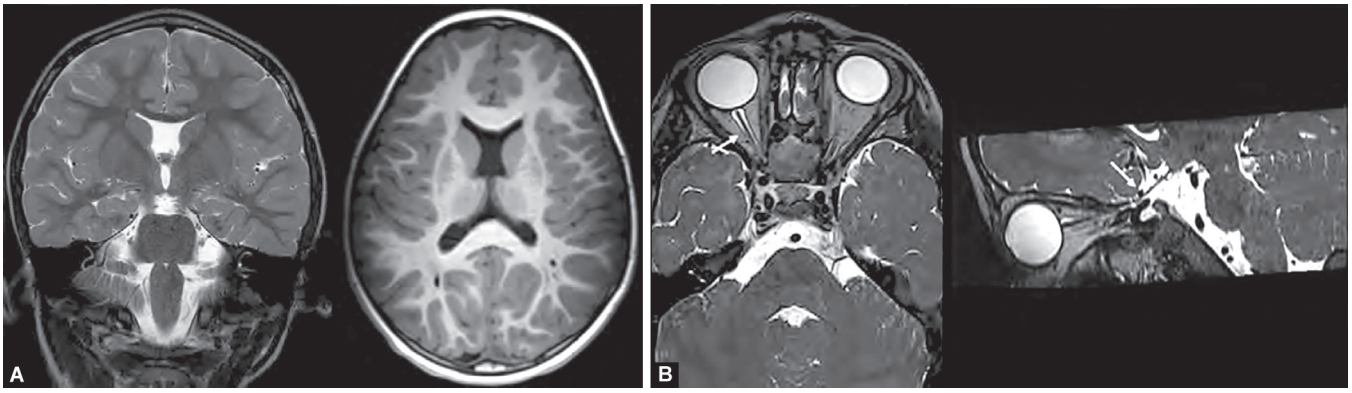
There are other environmental factors that might contribute to the risk of HPE. Maternal diabetes can increase the risk of HPE in the fetus.[117,118] Exposure to retinoic acid, diphenylhydantoin, aspirin, misoprostol, methotrexate, cholesterol-lowering agents, and alcohol during pregnancy have been associated with HPE.[46,92,119–123]

Clinical Features

- HPE is a malformation sequence with varying degrees of severity of brain and facial abnormalities.[1,124] As mentioned above, intellectual disability and seizures are seen in about 40% of all patients.[24] These seizures can sometimes be difficult to control. In some patients, seizures can be triggered by low blood glucose levels and/or hyponatremia secondary to defects in the pituitary gland.[5]
- Affected infants may show microcephaly or hydrocephalus (18%).[10] Neural tube defects occur in a small proportion of individuals.[125]
- Nearly all patients show altered muscle tone and developmental delay.[4,15] Those with alobar HPE have severe developmental delays.[126] In contrast, 50% of those with lobar HPE are able to ambulate with or without assistance, use their hands, and have some verbal communication.[4] Compared to receptive language and socialization, visual reasoning, and nonverbal problem skills are more severely afflicted.[5] Most people with non-syndromic HPE have developmental delays and intellectual disability.[10]
- Facial abnormalities are seen frequently. Some individuals with non-syndromic HPE have distinctive facial features, including a narrowing of the head at the temples, up slanting palpebral fissures, large ears, a short nose with upturned nostrils, and a broad philtrum.[26] The most severely affected patients may have cyclopia with a single central eye. Many show abnormalities in vision, which may be related to intrinsic eye problems or neurological problems in the CNS.[35] Nearly 80% of infants have unusual structures of the nose and mouth. Dental abnormalities such as a single central incisor and a cleft lip and/or palate are seen frequently. The sense of smell may be diminished or completely absent.[10] Microtia-anotia and other anomalies may also be seen.[127]
- Feeding difficulties are a major problem.[1] Hypothalamic and brain stem dysfunction result in axial hypotonia, poor suck-swallow reflex and coordination, frequent pauses, and vomiting with risk of aspiration.[5,10] Oral-sensory dysfunction may affect feeding especially when associated with textural aversion and labial and lingual weakness.[4,10] There is also instability of temperature, heart rate, and respiration. Some show altered gut peristalsis with abdominal bloating, and constipation.[4,10] Children with a cleft lip and/or palate often have additional difficulties with oral feeding.[4,10,15]
- Many patients show altered development of the hypothalamus and the pituitary gland with altered growth hormone levels and central diabetes insipidus. Thyroid and adrenal gland malfunction may be seen. Most people with non-syndromic HPE have a malfunctioning pituitary gland and frequently develop diabetes insipidus with altered fluid-electrolyte balance.[5,128] Some infants show altered autonomic function with abnormal breathing and temperature maintenance.[129]
- Some infants show genital abnormalities. Infants with dup(3p) syndrome show holoprosencephaly, characteristic facial changes, congenital heart defects, and hypoplasia of male genitalia.[130] In one study, 24% of the infants with HPE had genital abnormalities.[131]

Neuroimaging

The diagnosis of HPE is usually made by MRI or CT of the brain. HSP can sometimes be detected prenatally through ultrasound or MRI, though mild forms may not be reliably detected prenatally.[132–134]



Figs 15A and B: (A) Coronal T2 and axial T1-weighted MRI in a child with septo-optic dysplasia. The leaves of the septum pellucidum are lacking with resultant box-like configuration of the anterior lateral ventricles; (B) High-resolution axial and oblique sagittal T2-weighted MRI of the orbits on the same patient show a high-grade hypoplasia of the optic nerve (arrows)

Signs on Imaging

Butterfly sign: It is the visualization of the normal appearance of both choroid plexes in the axial plane on an antenatal ultrasound scan. Its absence suggests HPE.[135]

Absence of cavum septi pellucidi (CSP): Individuals with all types of HPE lack a normal CSP.[136]

"Snake under the skull" sign: The defective cortical tissue bridge between the 2 frontal gyri in lobar HPE pushes the anterior cerebral artery outside alongside the frontal bone.[4]

Other neuroimaging abnormalities include non-separation of deep gray nuclei, especially thalamus, fused single ventricle, dorsal cyst, hydrocephalus, Sylvian fissure abnormalities, and cortical dysplasia.[132,137]

Differential Diagnosis

Septo-optic dysplasia (SOD), also known as De Morsier syndrome: In addition to ventriculomegaly and failure of the hypothalamic-pituitary axis, these infants may also show optic nerve degeneration and absent septum pellucidum, is a condition characterized by optic nerve hypoplasia and absence of the septum pellucidum and, in two-thirds of patients hypothalamic-pituitary dysfunction.[138] Many experts believe that it is a part of the HPE spectrum.[139] The etiological associations overlap; similar to HPE, SOD is associated with maternal diabetes, in-utero exposure to quinidine and antiepileptics, alcohol, and cytomegalovirus infections (Fig. 15).[140]

MRI is the modality of choice for assessing SOD.[141] Findings may include a "point down" appearance of the lateral ventricular frontal horns on coronal images, absent septum pellucidum, hypoplastic pituitary stalk, hypoplastic optic chiasm/optic nerves and globes.[142]

DiGeorge syndrome: These patients show a cleft palate and hypertelorism, along with cardiopulmonary anomalies such as aortic arch and conotruncal defects.[143] Depending on the postnatal age, other abnormalities such as hypocalcemia and T-cell deficiency resulting from parathyroid hypoplasia may also be seen.

Hydranencephaly: Neuroimaging would show absent bilateral cerebral hemispheres with no cortical mantle and a fluid-filled cavity. However, HPE is excluded because the entire *falx cerebri* is preserved.[144]

Porencephalic cyst: Neuroimaging would show abnormal accumulation of CSF within the brain parenchyma, not the absence/midline fusion of cerebral lobes.[145,146]

Arachnoid cyst: These cysts can be an isolated finding or be seen in conjunction with HPE. It can occur following trauma or could be inherited in an autosomal manner. There is no facial dysmorphism.[72]

Approach to Genetic Diagnosis

The evaluation strategies to diagnose HPE in a proband include multiple steps.

Prenatal history to identify environmental causes: The most important teratogen known to cause HPE is maternal diabetes mellitus.[46] Both gestational and pre-gestational diabetes increase the risk, but insulin-dependent pre-gestational diabetes causes a 10-fold increase in the risk of HPE.[147,148]

Physical examination: As described above, a detailed clinical evaluation can be helpful in diagnosing syndromic forms of HPE. We can find extracranial anomalies in these patients.

Family history: A three-generation family history should be obtained, focusing on pregnancy loss, neonatal deaths, and relatives with HPE and/or developmental delay, including results of molecular genetic testing.[10]

Prenatal Testing and Preimplantation Genetic Testing

High-risk pregnancies

Molecular genetic testing: If HPE-causing pathogenic variant(s) have been identified in another affected family member, prenatal testing and preimplantation genetic testing are possible.

Fetal ultrasound examination: For families with non-syndromic HPE and no identifiable etiology, prenatal ultrasound examination can be helpful at 10–14 weeks of gestation and beyond.

Fetal MRI: Is a useful second-line investigation in several centers to evaluate CNS structures. Ultrafast MRI minimizes artifacts caused by fetal motion.

Low-risk pregnancies: When HPE is found on routine prenatal ultrasound examination in a fetus not known to be at increased risk for HPE, an extensive fetal examination using high-resolution ultrasound examination, MRI, fetal karyotype, and chromosomal

microarray, can be useful. Of note, alternative assays such as polymerase chain reaction-based multiplex ligation-dependent probe amplification can also be useful. Multigene panel sequencing including at least the three following genes: *SHH*, *ZIC2*, and *SIX3* can also be used.

Molecular Genetic Testing

We may need a combination of gene-targeted (multigene panel or single-gene testing) and comprehensive genomic testing (microarray analysis, genome sequencing, exome sequencing, and or exome array). Gene-targeted testing requires the clinician to hypothesize which gene(s) are likely involved, whereas genomic testing does not.

- Chromosomal microarray analysis (CMA) can detect genome-wide large deletions/duplications, including non-syndromic HPE and HPE with associated anomalies. CMA has been useful in identifying pathogenic variants in up to 14% of patients who have a normal karyotype and no causative gene(s) identified on multigene panel testing.[10]
- Chromosome analysis: Detection of numeric and structural cytogenetic abnormalities in HPE follows a series of steps like those used for other genetic conditions or birth defects. Recommended methods include G-banding karyotype and CMA.[149]
- Single-gene testing: When a specific syndromic cause of HPE is considered, the gene of interest can be sequenced to detect missense, nonsense, and splice site variants, and small intragenic deletions/insertions.[10]
- A multigene panel is most likely to identify the genetic cause of HPE but this approach limits identification of variants of uncertain significance. The genes included in the panel and the diagnostic sensitivity of these targets may change over time. The panels also may need modification based on ethnicity and/or geographic region of origin. The panel options may have to be customized. The methods may include sequence analysis, deletion/duplication analysis, and/or other non-sequencing-based tests.[150]
- Comprehensive genomic testing does not require the clinician to determine which gene(s) are likely involved.[151] Exome sequencing is used most frequently, but genome sequencing is also possible. If exome sequencing is not diagnostic, and if evidence supports AD or Mendelian inheritance, an exome array may have to be considered to detect multiexon deletions or duplications that cannot be detected by sequence analysis.

Management of the infant after birth: The management of these patients requires a multidisciplinary approach. Most of the steps in treatment involve symptomatic measures.[5,15,126]

- Seizures are seen frequently; nearly half of all patients will have at least one seizure. Some infants may require multidrug antiepileptic therapy.
- When macrocephaly is seen on examination, the patient should be evaluated for hydrocephalus. There may be a need for shunt surgery.
- Motor anomalies such as spasticity and dystonia may require pharmacological interventions, including intrathecal baclofen, oral trihexyphenidyl, and physical/occupational therapy.
- Orofacial motor dysfunction, particularly if there are structural anomalies such as cleft palate/lip, and/or functional abnormalities with gastroesophageal reflux and abnormal peristalsis can be a serious clinical challenge. This can require surgical corrections such as a gastrostomy tube. Medications and anti-reflux procedures may also be indicated.
- Endocrine dysregulation due to hypothalamic, pituitary, thyroid, and/or adrenal dysfunction may require specific evaluation and management. There might be a need to evaluate for diabetes insipidus. Some patients may also have growth hormone deficiency.

Life expectancy: Moderate-to-severe cases of HPE generally have a poor prognosis. The mortality rate increases with 33% of neonates dying in the first 24 hours after birth, 58% in the first month, 50% between the fourth and fifth months, and 70–80% in the first year of life.[4] The most severely afflicted infants with cyclopia or ethmocephaly are at risk of early mortality.[1]

In recent years, advances in diagnostic methods and clinical management have enabled a significant proportion of those with less severe disease to survive the past 12 months and beyond.[1,152] The survival rate after 1 year of life is approximately 29%.[25] Nearly 85% of these infants have a mild or non-classic facial phenotype. Major problems in these patients include sensorineural hearing loss (30%) and cortical vision impairment (20%).[10]

It is still important to remember that nearly half of the survivors to adolescence and later are non-ambulatory, non-verbal, and remain dependent on caregivers.[153] Seizures remain a major problem.[4] Hydrocephalus is another common issue, particularly in those with the alobar variant.[5]

Gastrointestinal tract anomalies like gastrointestinal reflux and gut dysmotility result from poor neuronal migration during development.[25]

Endocrine and sexual dysfunction/immaturity need management. Hormone irregularities of the posterior pituitary are seen more frequently than those of the anterior pituitary.[4]

Genetic counseling: Counseling families who have a child with HPE can be a difficult task.[154] We provide some guiding principles below, but the approach often needs to be individualized.

Chromosome Abnormality

Parents of a proband: (A) if the child has a numeric chromosome abnormality, the parents are expected to be normal; (B) if the child has a structural unbalanced chromosome rearrangement, they are at risk of having a balanced chromosome rearrangement and need evaluation.

Sibs of a proband: (A) if the child has a numeric chromosome abnormality, they are at a slightly increased risk of having a similar chromosome abnormality with a similar or different phenotype; (B) if the child has a structural unbalanced chromosome rearrangement, they are at no risk if neither parent has a structural rearrangement. The risk is increased if the parent has a balanced structural rearrangement, and needs evaluation.

Offspring of a proband: Individuals with HPE and a chromosome rearrangement are unlikely to reproduce.

Other family members: The risk to other family members depends on the status of the proband's parents: If a parent has a chromosome rearrangement, the parent's family members need evaluation.

AD Inheritance: The risk to family members may be higher as only one abnormal gene needs to be inherited from one parent.

Parents of a proband: If the patient has non-syndromic HPE, she/he may have inherited a pathogenic variant from a heterozygous parent who may or may not have HPE-spectrum anomalies. Some individuals with AD non-syndromic HPE may have the disorder as the result of a *de novo* pathogenic variant such as in the genes *SHH*, *ZIC2*, and *SIX3*. Molecular genetic testing is recommended for the parents of a proband.

Sibs of a proband: The risk to the sibs of the proband depends on the genetic status of the parents: If a parent is affected or has an HPE pathogenic variant (with or without clinical manifestations), the sibs need evaluation.

Offspring of a proband: Need evaluation.

Other family members: If a parent is affected or has a pathogenic variant, the parent's family members should be evaluated.

AR Inheritance

Parents of a proband: The parents of an affected child are obligated. Molecular genetic testing is recommended for the parents of a proband to confirm that both parents are heterozygous for a pathogenic variant and to allow reliable assessment of the risk of recurrence. Heterozygotes (carriers) are asymptomatic.

Sibs of a proband: If both parents are known to be heterozygous, each sib of an affected individual has at conception a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Heterozygotes (carriers) are asymptomatic.

Offspring of a proband: Depending on the gene, individuals may not be able to reproduce.

Other family members: Each sib of the proband's parents is at a 50% risk of being a carrier.

X-Linked Inheritance

Parents of a female proband: A female proband with X-linked HPE may have inherited a pathogenic variant from either her mother or her father, or the pathogenic variant may be *de novo*. Molecular genetic testing is recommended for both parents of a female proband.

Parents of a male proband: If a male is the only affected family member, the mother may be heterozygous or the affected male may have a *de novo* pathogenic variant, in which case the mother is not heterozygous. Molecular genetic testing is recommended for the mother of a male proband.

Sibs of a female proband: The risk to sibs depends on the genetic status of the parents. If the mother has a pathogenic variant, the chance of transmitting it in each pregnancy is 50%. If the father has a pathogenic variant, he will transmit it to his daughters and none of his sons.

Sibs of a male proband: The risk to sibs depends on the genetic status of the mother: The chance of transmitting it in each pregnancy may be up to 50%.

Offspring of a proband: Each child of a female proband with X-linked HPE has a 50% chance of inheriting the pathogenic variant. Females are unlikely to reproduce. Each daughter of a male proband with X-linked HPE has a 50% chance of inheriting a pathogenic variant. Surviving male probands with HPE are unlikely to reproduce.

Other family members: The risk to other family members depends on the genetic status of the proband's parents. Need testing.

References

1. Dubourg C, Bendavid C, Pasquier L, et al. Holoprosencephaly. Orphanet J Rare Dis 2007;2:8. DOI: 10.1186/1750-1172-2-8.
2. Delezoide AL, Narcy F, Larroche JC. Cerebral midline developmental anomalies: Spectrum and associated features. Genet Couns 1990;1(3-4):197–210. PMID: 2098044.
3. Volpe P, Campobasso G, De Robertis V, et al. Disorders of prosencephalic development. Prenat Diagn 2009;29(4):340–354. DOI: 10.1002/pd.2208.
4. Ramakrishnan S, Gupta V. Holoprosencephaly. StatPearls. Treasure Island (FL). 2023.
5. Raam MS, Solomon BD, Muenke M. Holoprosencephaly: A guide to diagnosis and clinical management. Indian Pediatr 2011;48(6):457–466. DOI: 10.1007/s13312-011-0078-x.
6. Muhr J, Arbor TC, Ackerman KM. Embryology, gastrulation. StatPearls. Treasure Island (FL). 2023.
7. Mishra S, Sabhlok S, Panda PK, et al. Management of midline facial clefts. J Maxillofac Oral Surg 2015;14(4):883–890. DOI: 10.1007/s12663-015-0763-8.
8. Kobori JA, Herrick MK, Urich H. Arhinencephaly. The spectrum of associated malformations. Brain 1987;110(1):237–260. DOI: 10.1093/brain/110.1.237.
9. Parizad N, Faraji N, Hassanpour A, et al. Cyclopia, a newborn with a single eye, a rare but lethal congenital anomaly: A case report. Int J Surg Case Rep 2021;88:106548. DOI: 10.1016/j.ijscr.2021.106548.
10. Tekendo-Ngongang C, Muenke M, Kruszka P. Holoprosencephaly overview. In: Adam MP, Feldman J, Mirzaa GM, editors. GeneReviews®. Seattle (WA): University of Washington, Seattle. 1993–2023.
11. Jaramillo C, Brandt SK, Jorgenson RJ. Autosomal dominant inheritance of the DeMyer Sequence. J Craniofac Genet Dev Biol 1988;8(3):199–204. PMID: 3209682.
12. Wilson SW, Houart C. Early steps in the development of the forebrain. Dev Cell 2004;6(2):167–181. DOI: 10.1016/s1534-5807(04)00027-9.
13. Roth DM, Bayona F, Baddam P, et al. Craniofacial development: Neural crest in molecular embryology. Head Neck Pathol 2021;15(1):1–15. DOI: 10.1007/s12105-021-01301-z.
14. Ewings EL, Carstens MH. Neuroembryology and functional anatomy of craniofacial clefts. Indian J Plast Surg 2009;42 Suppl(Suppl):S19–S34. DOI: 10.4103/0970-0358.57184.
15. Kauvar EF, Muenke M. Holoprosencephaly: Recommendations for diagnosis and management. Curr Opin Pediatr 2010;22(6):687–695. DOI: 10.1097/MOP.0b013e32833f56d5.
16. Oba H, Barkovich AJ. Holoprosencephaly: An analysis of callosal formation and its relation to development of the interhemispheric fissure. AJNR Am J Neuroradiol 1995;16(3):453–460. PMID: 7793363.
17. Orioli IM, Amar E, Bakker MK, et al. Cyclopia: An epidemiologic study in a large dataset from the International Clearinghouse of Birth Defects Surveillance and Research. Am J Med Genet C Semin Med Genet 2011;157C(4):344–357. DOI: 10.1002/ajmg.c.30323.
18. Gondre-Lewis MC, Gboluaje T, Reid SN, et al. The human brain and face: Mechanisms of cranial, neurological and facial development revealed through malformations of holoprosencephaly, cyclopia and aberrations in chromosome 18. J Anat 2015;227(3):255–267. DOI: 10.1111/joa.12343.
19. Kunwar A, Shrestha BM, Shrestha S, et al. Cyclopia with proboscis: A rare congenital anomaly. Clin Case Rep 2021;9(7):e04466. DOI: 10.1002/ccr3.4466.
20. Dewan P, Rohatgi S, Roy S, et al. Ethmocephaly: A rare cephalic disorder. J Pediatr Neurosci 2016;11(1):92–93. DOI: 10.4103/1817-1745.181262.

21. Lin CH, Tsai JD, Ho YJ, et al. Alobar holoprosencephaly associated with cebocephaly and craniosynostosis. Acta Neurol Taiwan 2009;18(2):123–126. PMID: 19673365.
22. Verma AS, Fitzpatrick DR. Anophthalmia and microphthalmia. Orphanet J Rare Dis 2007;2:47. DOI: 10.1186/1750-1172-2-47.
23. Lakhkar BB, Lakhkar BN, Lakhkar BB. Semilobarholoprosencephaly-A dreading congenital anomaly. J Clin Diagn Res 2017;11(6):TD03–TD04. DOI: 10.7860/JCDR/2017/26530.10105.
24. Nonkulovski D, Sofijanova A, Spasovska T, et al. Semilobar holoprosencephaly caused by a novel and De Novo ZIC2 pathogenic variant. Balkan J Med Genet 2023;25(2):71–76. DOI: 10.2478/bjmg-2022-0017.
25. Malta M, AlMutiri R, Martin CS, et al. Holoprosencephaly: Review of embryology, clinical phenotypes, etiology and management. Children (Basel) 2023;10(4):647. DOI: 10.3390/children10040647.
26. Honey EM, Butow KW, Zwahlen RA. Holoprosencephaly with Clefts: Data of 85 patients, treatment and outcome: Part 1: History, subdivisions, and data on 85 holoprosencephalic cleft patients. Ann Maxillofac Surg 2019;9(1):140–145. DOI: 10.4103/ams.ams_50_19.
27. Pilu G, Ambrosetto P, Sandri F, et al. Intraventricular fused fornices: A specific sign of fetal lobar holoprosencephaly. Ultrasound Obstet Gynecol 1994;4(1):65–67. DOI: 10.1046/j.1469-0705.1994.04010065.x.
28. Rajalakshmi PP, Gadodia A, Priyatharshini P. Middle interhemispheric variant of holoprosencephaly: A rare midline malformation. J Pediatr Neurosci 2015;10(3):244–246. DOI: 10.4103/1817-1745.165678.
29. Bulakbasi N, Cancuri O, Kocaoglu M. The middle interhemispheric variant of holoprosencephaly: Magnetic resonance and diffusion tensor imaging findings. Br J Radiol 2016;89(1063):20160115. DOI: 10.1259/bjr.20160115.
30. Oegema R, Barkovich AJ, Mancini GMS, et al. Subcortical heterotopic gray matter brain malformations: Classification study of 107 individuals. Neurology 2019;93(14):e1360–e1373. DOI: 10.1212/WNL.0000000000008200.
31. Fallet-Bianco C. Neuropathology of holoprosencephaly. Am J Med Genet C Semin Med Genet 2018;178(2):214–228. DOI: 10.1002/ajmg.c.31623.
32. Simon EM, Hevner RF, Pinter JD, et al. The middle interhemispheric variant of holoprosencephaly. AJNR Am J Neuroradiol 2002;23(1):151–156. PMID: 11827888.
33. Severino M, Geraldo AF, Utz N, et al. Definitions and classification of malformations of cortical development: Practical guidelines. Brain 2020;143(10):2874–2894. DOI: 10.1093/brain/awaa174.
34. Hahn JS, Barnes PD, Clegg NJ, et al. Septopreoptic holoprosencephaly: A mild subtype associated with midline craniofacial anomalies. AJNR Am J Neuroradiol 2010;31(9):1596–1601. DOI: 10.3174/ajnr.A2123.
35. Pineda-Alvarez DE, Solomon BD, Roessler E, et al. A broad range of ophthalmologic anomalies is part of the holoprosencephaly spectrum. Am J Med Genet A 2011;155A(11):2713–2720. DOI: 10.1002/ajmg.a.34261.
36. Thomas EM, Gibikote S, Panwar JS, et al. Congenital nasal pyriform aperture stenosis: A rare cause of nasal airway obstruction in a neonate. Indian J Radiol Imaging 2010;20(4):266–268. DOI: 10.4103/0971-3026.73539.
37. Hall RK. Solitary median maxillary central incisor (SMMCI) syndrome. Orphanet J Rare Dis 2006;1:12. DOI: 10.1186/1750-1172-1-12.
38. Negi A, Negi A, Mohanan M. Solitary median maxillary central incisor syndrome: A rare entity. J Oral Maxillofac Pathol 2020;24(2):402. DOI: 10.4103/jomfp.JOMFP_183_19.
39. Blackmore K, Wynne DM. A case of solitary median maxillary central incisor (SMMCI) syndrome with bilateral pyriform aperture stenosis and choanal atresia. Int J Pediatr Otorhinolaryngol 2010;74(8):967–969. DOI: 10.1016/j.ijporl.2010.05.018.
40. Sharma S, Sharma V, Bothra M. Frontonasal dysplasia (Median cleft face syndrome). J Neurosci Rural Pract 2012;3(1):65–67. DOI: 10.4103/0976-3147.91947.
41. Lee SI, Lee SJ, Joo HS. Frontonasal dysplasia: A case report. Arch Craniofac Surg 2019;20(6):397–400. DOI: 10.7181/acfs.2019.00570.
42. Farlie PG, Baker NL, Yap P, et al. Frontonasal dysplasia: Towards an understanding of molecular and developmental aetiology. Mol Syndromol 2016;7(6):312–321. DOI: 10.1159/000450533.
43. Martinelli P, Russo R, Agangi A, et al. Prenatal ultrasound diagnosis of frontonasal dysplasia. Prenat Diagn 2002;22(5):375–379. DOI: 10.1002/pd.287.
44. Richieri-Costa A, Guion-Almeida ML. The syndrome of frontonasal dysplasia, callosal agenesis, basal encephalocele, and eye anomalies-Phenotypic and aetiological considerations. Int J Med Sci 2004;1(1):34–42. DOI: 10.7150/ijms.1.34.
45. Meryem B, Amine N, Houssein O, et al. Antenatal and postnatal diagnosis of semilobar holoprosencephaly: Two case reports. Glob Pediatr Health 2023;10:2333794X231156037. DOI: 10.1177/2333794X231156037.
46. Summers AD, Reefhuis J, Taliano J, et al. Nongenetic risk factors for holoprosencephaly: An updated review of the epidemiologic literature. Am J Med Genet C Semin Med Genet 2018;178(2):151–164. DOI: 10.1002/ajmg.c.31614.
47. Kruszka P, Muenke M. Syndromes associated with holoprosencephaly. Am J Med Genet C Semin Med Genet 2018;178(2):229–237. DOI: 10.1002/ajmg.c.31620.
48. Geng X, Oliver G. Pathogenesis of holoprosencephaly. J Clin Invest 2009;119(6):1403–1413. DOI: 10.1172/JCI38937.
49. Costa AD, Schultz R, Rosemberg S. Alobar holoprosencephaly and Trisomy 13 (Patau syndrome). Autops Case Rep 2013;3(2):5–10. DOI: 10.4322/acr.2013.012.
50. Swatek J, Szumilo J, Burdan F. Alobar holoprosencephaly with cyclopia-autopsy-based observations from one medical center. Reprod Toxicol 2013;41:80–85. DOI: 10.1016/j.reprotox.2013.06.060.
51. Hu T, Kruszka P, Martinez AF, et al. Cytogenetics and holoprosencephaly: A chromosomal microarray study of 222 individuals with holoprosencephaly. Am J Med Genet C Semin Med Genet 2018;178(2):175–186. DOI: 10.1002/ajmg.c.31622.
52. Kelley RL, Roessler E, Hennekam RC, et al. Holoprosencephaly in RSH/smith-lemli-opitz syndrome: Does abnormal cholesterol metabolism affect the function of Sonic Hedgehog? Am J Med Genet 1996;66(4):478–484. DOI: 10.1002/(SICI)1096-8628(19961230)66:4<478::AID-AJMG22>3.0.CO;2-Q.
53. Kelley RI, Hennekam RC. The smith-lemli-opitz syndrome. J Med Genet 2000;37(5):321–335. DOI: 10.1136/jmg.37.5.321.
54. DeBarber AE, Eroglu Y, Merkens LS, et al. Smith-lemli-opitz syndrome. Expert Rev Mol Med 2011;13:e24. DOI: 10.1017/S146239941100189X.
55. Simonis N, Migeotte I, Lambert N, et al. FGFR1 mutations cause Hartsfield syndrome, the unique association of holoprosencephaly and ectrodactyly. J Med Genet 2013;50(9):585–592. DOI: 10.1136/jmedgenet-2013-101603.
56. Herman S, Delio M, Morrow B, et al. Agnathia-otocephaly complex: A case report and examination of the OTX2 and PRRX1 genes. Gene 2012;494(1):124–129. DOI: 10.1016/j.gene.2011.11.033.
57. Biesecker LG. GLI3-Related Pallister-Hall Syndrome. In: Adam MP, Feldman J, Mirzaa GM, et al., (Eds). GeneReviews((R)). Seattle (WA). 1993.
58. Bae GU, Domene S, Roessler E, et al. Mutations in CDON, encoding a hedgehog receptor, result in holoprosencephaly and defective interactions with other hedgehog receptors. Am J Hum Genet 2011;89(2):231–240. DOI: 10.1016/j.ajhg.2011.07.001.
59. Travessa A, Dias P, Rocha P, et al. Prenatal diagnosis of holoprosencephaly associated with smith-lemli-opitz syndrome (SLOS) in a 46,XX fetus. Taiwan J Obstet Gynecol 2017;56(4):541–544. DOI: 10.1016/j.tjog.2017.01.012.
60. Ionescu CA, Calin D, Navolan D, et al. Alobar holoprosencephaly associated with a rare chromosomal abnormality: Case report and literature review. Medicine (Baltimore) 2018;97(29):e11521. DOI: 10.1097/MD.0000000000011521.
61. Vaaralahti K, Raivio T, Koivu R, et al. Genetic overlap between holoprosencephaly and Kallmann syndrome. Mol Syndromol 2012;3(1):1–5. DOI: 10.1159/000338706.

62. Cole LW, Sidis Y, Zhang C, et al. Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: Molecular genetics and clinical spectrum. J Clin Endocrinol Metab 2008;93(9):3551–3559. DOI: 10.1210/jc.2007-2654.
63. Correa FA, Trarbach EB, Tusset C, et al. FGFR1 and PROKR2 rare variants found in patients with combined pituitary hormone deficiencies. Endocr Connect 2015;4(2):100–107. DOI: 10.1530/EC-15-0015.
64. Mimaki M, Shiihara T, Watanabe M, et al. Holoprosencephaly with cerebellar vermis hypoplasia in 13q deletion syndrome: Critical region for cerebellar dysgenesis within 13q32.2q34. Brain Dev 2015;37(7):714–718. DOI: 10.1016/j.braindev.2014.10.009.
65. Jin Q, Qiang R, Cai B, et al. The genotype and phenotype of chromosome 18p deletion syndrome: Case series. Medicine (Baltimore) 2021;100(18):e25777. DOI: 10.1097/MD.0000000000025777.
66. Temple SEL, Sachdev R, Ellaway C. Familial DHCR7 genotype presenting as a very mild form of Smith-Lemli-Opitz syndrome and lethal holoprosencephaly. JIMD Rep 2020;56(1):3–8. DOI: 10.1002/jmd2.12155.
67. Jones GE, Robertson L, Maniyar A, et al. Microform holoprosencephaly with bilateral congenital elbow dislocation; increasing the phenotypic spectrum of Steinfeld syndrome. Am J Med Genet A 2016;170(3):754–759. DOI: 10.1002/ajmg.a.37511.
68. Valenza F, Cittaro D, Stupka E, et al. A novel truncating variant of GLI2 associated with Culler-Jones syndrome impairs Hedgehog signalling. PLoS One 2019;14(1):e0210097. DOI: 10.1371/journal.pone.0210097.
69. Richieri-Costa A, Vendramini-Pittoli S, Kokitsu-Nakata NM, et al. Multisystem involvement in a patient with a PTCH1 mutation: Clinical and imaging findings. J Pediatr Genet 2017;6(2):103–106. DOI: 10.1055/s-0036-1588028.
70. Loo CKC, Pearen MA, Ramm GA. The role of sonic hedgehog in human holoprosencephaly and short-rib polydactyly syndromes. Int J Mol Sci 2021;22(18):9854. DOI: 10.3390/ijms22189854.
71. Roessler E, Muenke M. The molecular genetics of holoprosencephaly. Am J Med Genet C Semin Med Genet 2010;154C(1):52–61. DOI: 10.1002/ajmg.c.30236.
72. Abramyan J. Hedgehog signaling and embryonic craniofacial disorders. J Dev Biol 2019;7(2). DOI: 10.3390/jdb7020009.
73. Petryk A, Graf D, Marcucio R. Holoprosencephaly: Signaling interactions between the brain and the face, the environment and the genes, and the phenotypic variability in animal models and humans. Wiley Interdiscip Rev Dev Biol 2015;4(1):17–32. DOI: 10.1002/wdev.161.
74. Keaton AA, Solomon BD, Kauvar EF, et al. TGIF mutations in human holoprosencephaly: Correlation between genotype and phenotype. Mol Syndromol 2010;1(5):211–222. DOI: 10.1159/000328203.
75. Wallis DE, Roessler E, Hehr U, et al. Mutations in the homeodomain of the human SIX3 gene cause holoprosencephaly. Nat Genet 1999;22(2):196–198. DOI: 10.1038/9718.
76. Reinders MG, van Hout AF, Cosgun B, et al. New mutations and an updated database for the patched-1 (PTCH1) gene. Mol Genet Genomic Med 2018;6(3):409–415. DOI: 10.1002/mgg3.380.
77. Roessler E, Ouspenskaia MV, Karkera JD, et al. Reduced NODAL signaling strength via mutation of several pathway members including FOXH1 is linked to human heart defects and holoprosencephaly. Am J Hum Genet 2008;83(1):18–29. DOI: 10.1016/j.ajhg.2008.05.012.
78. Bashamboo A, Bignon-Topalovic J, Rouba H, et al. A nonsense mutation in the hedgehog receptor CDON associated with pituitary stalk interruption syndrome. J Clin Endocrinol Metab 2016;101(1):12–15. DOI: 10.1210/jc.2015-2995.
79. Arauz RF, Solomon BD, Pineda-Alvarez DE, et al. A hypomorphic allele in the FGF8 gene contributes to holoprosencephaly and is allelic to gonadotropin-releasing hormone deficiency in humans. Mol Syndromol 2010;1(2):59–66. DOI: 10.1159/000302285.
80. Ishiguro A, Hatayama M, Otsuka MI, et al. Link between the causative genes of holoprosencephaly: Zic2 directly regulates Tgif1 expression. Sci Rep 2018;8(1):2140. DOI: 10.1038/s41598-018-20242-2.
81. Zhang XM, Yang XJ. Temporal and spatial effects of Sonic hedgehog signaling in chick eye morphogenesis. Dev Biol 2001;233(2):271–290. DOI: 10.1006/dbio.2000.0195.
82. Diacou R, Zhao Y, Zheng D, et al. Six3 and Six6 are jointly required for the maintenance of multipotent retinal progenitors through both positive and negative regulation. Cell Rep 2018;25(9):2510–2523 e4. DOI: 10.1016/j.celrep.2018.10.106.
83. Nanni L, Ming JE, Bocian M, et al. The mutational spectrum of the sonic hedgehog gene in holoprosencephaly: SHH mutations cause a significant proportion of autosomal dominant holoprosencephaly. Hum Mol Genet 1999;8(13):2479–2488. DOI: 10.1093/hmg/8.13.2479.
84. Singh S, Tokhunts R, Baubet V, et al. Sonic hedgehog mutations identified in holoprosencephaly patients can act in a dominant negative manner. Hum Genet 2009;125(1):95–103. DOI: 10.1007/s00439-008-0599-0.
85. Xiong J, Xiang B, Chen X, et al. Case report: A novel mutation in ZIC2 in an infant with microcephaly, holoprosencephaly, and arachnoid cyst. Medicine (Baltimore) 2019;98(10):e14780. DOI: 10.1097/MD.0000000000014780.
86. Roessler E, Lacbawan F, Dubourg C, et al. The full spectrum of holoprosencephaly-associated mutations within the ZIC2 gene in humans predicts loss-of-function as the predominant disease mechanism. Hum Mutat 2009;30(4):E541–E554. DOI: 10.1002/humu.20982.
87. Tasdemir S, Sahin I, Cayir A, et al. Holoprosencephaly: ZIC2 mutation in a case with panhypopituitarism. J Pediatr Endocrinol Metab 2014;27(7-8):777–781. DOI: 10.1515/jpem-2013-0449.
88. Solomon BD, Lacbawan F, Mercier S, et al. Mutations in ZIC2 in human holoprosencephaly: Description of a novel ZIC2 specific phenotype and comprehensive analysis of 157 individuals. J Med Genet 2010;47(8):513–524. DOI: 10.1136/jmg.2009.073049.
89. Gounongbe C, Marangoni M, Gouder de Beauregard V, et al. Middle interhemispheric variant of holoprosencephaly: First prenatal report of a ZIC2 missense mutation. Clin Case Rep 2020;8(7):1287–1292. DOI: 10.1002/ccr3.2896.
90. Lacbawan F, Solomon BD, Roessler E, et al. Clinical spectrum of SIX3-associated mutations in holoprosencephaly: Correlation between genotype, phenotype and function. J Med Genet 2009;46(6):389–398. DOI: 10.1136/jmg.2008.063818.
91. Paulussen AD, Schrander-Stumpel CT, Tserpelis DC, et al. The unfolding clinical spectrum of holoprosencephaly due to mutations in SHH, ZIC2, SIX3 and TGIF genes. Eur J Hum Genet 2010;18(9):999–1005. DOI: 10.1038/ejhg.2010.70.
92. Hong M, Christ A, Christa A, et al. Cdon mutation and fetal alcohol converge on Nodal signaling in a mouse model of holoprosencephaly. Elife 2020;9:e60351. DOI: 10.7554/eLife.60351.
93. Kruszka P, Berger SI, Weiss K, et al. A CCR4-NOT Transcription Complex, Subunit 1, CNOT1, Variant Associated with Holoprosencephaly. Am J Hum Genet 2019;104(5):990–993. DOI: 10.1016/j.ajhg.2019.03.017.
94. Roessler E, Ma Y, Ouspenskaia MV, et al. Truncating loss-of-function mutations of DISP1 contribute to holoprosencephaly-like microform features in humans. Hum Genet 2009;125(4):393–400. DOI: 10.1007/s00439-009-0628-7.
95. Dupe V, Rochard L, Mercier S, et al. NOTCH, a new signaling pathway implicated in holoprosencephaly. Hum Mol Genet 2011;20(6):1122–1131. DOI: 10.1093/hmg/ddq556.
96. Tekendo-Ngongang C, Kruszka P, Martinez AF, et al. Novel heterozygous variants in KMT2D associated with holoprosencephaly. Clin Genet 2019;96(3):266–270. DOI: 10.1111/cge.13598.
97. Hughes JJ, Alkhunaizi E, Kruszka P, et al. Loss-of-function variants in PPP1R12A: From isolated sex reversal to holoprosencephaly spectrum and urogenital malformations. Am J Hum Genet 2020;106(1):121–128. DOI: 10.1016/j.ajhg.2019.12.004.
98. Goel H, Parasivam G. Another case of holoprosencephaly associated with RAD21 loss-of-function variant. Brain 2020;143(8):e64. DOI: 10.1093/brain/awaa173.

99. Kruszka P, Berger SI, Casa V, et al. Cohesin complex-associated holoprosencephaly. Brain 2019;142(9):2631–2643. DOI: 10.1093/brain/awz210.
100. Mouden C, de Tayrac M, Dubourg C, et al. Homozygous STIL mutation causes holoprosencephaly and microcephaly in two siblings. PLoS One 2015;10(2):e0117418. DOI: 10.1371/journal.pone.0117418.
101. Seppala M, Depew MJ, Martinelli DC, et al. Gas1 is a modifier for holoprosencephaly and genetically interacts with sonic hedgehog. J Clin Invest 2007;117(6):1575–1584. DOI: 10.1172/JCI32032.
102. Houtmeyers R, Tchouate Gainkam O, Glanville-Jones HA, et al. Zic2 mutation causes holoprosencephaly via disruption of NODAL signalling. Hum Mol Genet 2016;25(18):3946–3959. DOI: 10.1093/hmg/ddw235.
103. Ming JE, Kaupas ME, Roessler E, et al. Mutations in PATCHED-1, the receptor for SONIC HEDGEHOG, are associated with holoprosencephaly. Hum Genet 2002;110(4):297–301. DOI: 10.1007/s00439-002-0695-5.
104. Dubourg C, Carre W, Hamdi-Roze H, et al. Mutational spectrum in holoprosencephaly shows that FGF is a new major signaling pathway. Hum Mutat 2016;37(12):1329–1339. DOI: 10.1002/humu.23038.
105. McKean DM, Niswander L. Defects in GPI biosynthesis perturb Cripto signaling during forebrain development in two new mouse models of holoprosencephaly. Biol Open 2012;1(9):874–883. DOI: 10.1242/bio.20121982.
106. Bear KA, Solomon BD, Antonini S, et al. Pathogenic mutations in GLI2 cause a specific phenotype that is distinct from holoprosencephaly. J Med Genet 2014;51(6):413–418. DOI: 10.1136/jmedgenet-2013-102249.
107. Kordass U, Schroder C, Elbracht M, et al. A familial GLI2 deletion (2q14.2) not associated with the holoprosencephaly syndrome phenotype. Am J Med Genet A 2015;167A(5):1121–1124. DOI: 10.1002/ajmg.a.36972.
108. Martinez-Frias ML, Bermejo E, Garcia A, et al. Holoprosencephaly associated with caudal dysgenesis: A clinical-epidemiological analysis. Am J Med Genet 1994;53(1):46–51. DOI: 10.1002/ajmg.1320530110.
109. Morichon-Delvallez N, Delezoide AL, Vekemans M. Holoprosencephaly and sacral agenesis in a fetus with a terminal deletion 7q36-->7qter. J Med Genet 1993;30(6):521–524. DOI: 10.1136/jmg.30.6.521.
110. Cohen MM Jr, Gorlin RJ. Pseudo-trisomy 13 syndrome. Am J Med Genet 1991;39(3):332–335; discussion 336–337. DOI: 10.1002/ajmg.1320390316.
111. Utine GE, Alanay Y, Aktas D, et al. Pseudo-trisomy 13 in a fetus: further support for autosomal recessive inheritance. Turk J Pediatr 2008;50(3):287–290. PMID: 18773678.
112. Hacihamdioglu B, Siklar Z, Savas Erdeve S, et al. Genoa syndrome and central diabetes insipidus: a case report. J Clin Res Pediatr Endocrinol 2010;2(2):89–91. DOI: 10.4274/jcrpe.v2i2.89.
113. Camera G, Lituania M, Cohen MM Jr. Holoprosencephaly and primary craniosynostosis: The Genoa syndrome. Am J Med Genet 1993;47(8):1161–1165. DOI: 10.1002/ajmg.1320470806.
114. Kariminejad A, Goodarzi P, Asghari-Roodsari A, et al. Amelia, cleft lip, and holoprosencephaly: A distinct entity. Am J Med Genet A 2009;149A(12):2828–2831. DOI: 10.1002/ajmg.a.32933.
115. Thomas M, Donnai D. Bilateral brachial amelia with facial clefts and holoprosencephaly. Clinical Dysmorphology 1994;3(3):266–269. PMID: 7981864..
116. Sun Y, Ye X, Fan Y, et al. High detection rate of copy number variations using capture sequencing data: A retrospective study. Clin Chem 2020;66(3):455–462. DOI: 10.1093/clinchem/hvz033.
117. Barr M Jr, Hanson JW, Currey K, et al. Holoprosencephaly in infants of diabetic mothers. J Pediatr 1983;102(4):565–568. DOI: 10.1016/s0022-3476(83)80185-1.
118. Mishra V, Panigrahi N, Rao A, et al. Neurological abnormalities in infants of mothers with diabetes mellitus. Newborn (Clarksville) 2022;1(2):238–244. DOI: 10.5005/jp-journals-11002-0033.
119. Haas D, Muenke M. Abnormal sterol metabolism in holoprosencephaly. Am J Med Genet C Semin Med Genet 2010;154C(1):102–108. DOI: 10.1002/ajmg.c.30243.
120. Billington CJ Jr, Schmidt B, Marcucio RS, et al. Impact of retinoic acid exposure on midfacial shape variation and manifestation of holoprosencephaly in Twsg1 mutant mice. Dis Model Mech 2015;8(2):139–146. DOI: 10.1242/dmm.018275.
121. Kotzot D, Weigl J, Huk W, et al. Hydantoin syndrome with holoprosencephaly: A possible rare teratogenic effect. Teratology 1993;48(1):15–19. DOI: 10.1002/tera.1420480105.
122. Pirmez R, Freitas ME, Gasparetto EL, et al. Moebius syndrome and holoprosencephaly following exposure to misoprostol. Pediatr Neurol 2010;43(5):371–373. DOI: 10.1016/j.pediatrneurol.2010.05.026.
123. Seidahmed MZ, Shaheed MM, Abdulbasit OB, et al. A case of methotrexate embryopathy with holoprosencephaly, expanding the phenotype. Birth Defects Res A Clin Mol Teratol 2006;76(2):138–142. DOI: 10.1002/bdra.20199.
124. Aruna E, Chakravarthy VK, Rao DN, et al. Holoprosencephaly with multiple anomalies of the craniofacial bones-an autopsy report. J Clin Diagn Res 2013;7(8):1722–1724. DOI: 10.7860/JCDR/2013/5734.3268.
125. Bonneau D, Marechaud M, Odent S, et al. Heterotaxy-neural tube defect and holoprosencephaly occurring independently in two sib fetuses. Am J Med Genet 1999;84(4):373–376. DOI: 10.1002/(sici)1096-8628(19990604)84:4<373::aid-ajmg13>3.0.co;2-8.
126. Levey EB, Stashinko E, Clegg NJ, et al. Management of children with holoprosencephaly. Am J Med Genet C Semin Med Genet 2010;154C(1):183–190. DOI: 10.1002/ajmg.c.30254.
127. Mastroiacovo P, Corchia C, Botto LD, et al. Epidemiology and genetics of microtia-anotia: A registry based study on over one million births. J Med Genet 1995;32(6):453–457. DOI: 10.1136/jmg.32.6.453.
128. Hahn JS, Hahn SM, Kammann H, et al. Endocrine disorders associated with holoprosencephaly. J Pediatr Endocrinol Metab 2005;18(10):935–941. DOI: 10.1515/jpem.2005.18.10.935.
129. Gupta AO, Leblanc P, Janumpally KC, et al. A preterm infant with semilobar holoprosencephaly and hydrocephalus: A case report. Cases J 2010;3:35. DOI: 10.1186/1757-1626-3-35.
130. Martin NJ, Steinberg BG. The dup(3)(p25 leads to pter) syndrome: A case with holoprosencephaly. Am J Med Genet 1983;14(4):767–772. DOI: 10.1002/ajmg.1320140418.
131. Orioli IM, Castilla EE. Epidemiology of holoprosencephaly: Prevalence and risk factors. Am J Med Genet C Semin Med Genet 2010;154C(1):13–21. DOI: 10.1002/ajmg.c.30233.
132. Hahn JS, Barnes PD. Neuroimaging advances in holoprosencephaly: Refining the spectrum of the midline malformation. Am J Med Genet C Semin Med Genet 2010;154C(1):120–132. DOI: 10.1002/ajmg.c.30238.
133. Joo GJ, Beke A, Papp C, et al. Prenatal diagnosis, phenotypic and obstetric characteristics of holoprosencephaly. Fetal Diagn Ther 2005;20(3):161–166. DOI: 10.1159/000083897.
134. Mercier S, Dubourg C, Belleguic M, et al. Genetic counseling and "molecular" prenatal diagnosis of holoprosencephaly (HPE). Am J Med Genet C Semin Med Genet 2010;154C(1):191–196. DOI: 10.1002/ajmg.c.30246.
135. Sepulveda W, Dezerega V, Be C. First-trimester sonographic diagnosis of holoprosencephaly: value of the "butterfly" sign. J Ultrasound Med 2004;23(6):761–765; quiz 6–7. DOI: 10.7863/jum.2004.23.6.761.
136. Ben M'Barek I, Tassin M, Guet A, et al. Antenatal diagnosis of absence of septum pellucidum. Clin Case Rep 2020;8(3):498–503. DOI: 10.1002/ccr3.2666.
137. Plawner LL, Delgado MR, Miller VS, et al. Neuroanatomy of holoprosencephaly as predictor of function: Beyond the face predicting the brain. Neurology 2002;59(7):1058–1066. DOI: 10.1212/wnl.59.7.1058.
138. Reis P, Mourao J. Septo-optic dysplasia/de Morsier's syndrome. Saudi J Anaesth 2017;11(1):106–107. DOI: 10.4103/1658-354X.197350.
139. Fitz CR. Holoprosencephaly and septo-optic dysplasia. Neuroimaging Clin N Am 1994;4(2):263–281. PMID: 8081628.
140. Pilliod RA, Pettersson DR, Gibson T, et al. Diagnostic accuracy and clinical outcomes associated with prenatal diagnosis of fetal absent cavum septi pellucidi. Prenat Diagn 2018;38(6):395–401. DOI: 10.1002/pd.5247.

141. Wang CY, Ginat DT. Neuroimaging of septo-optic dysplasia-plus with midbrain hypoplasia and ophthalmoplegia. eNeurologicalSci 2020;19:100235. DOI: 10.1016/j.ensci.2020.100235.
142. Ward DJ, Connolly DJA, Griffiths PD. Review of the MRI brain findings of septo-optic dysplasia. Clin Radiol 2021;76(2):160 e1–e14. DOI: 10.1016/j.crad.2020.09.007.
143. Altshuler E, Saidi A, Budd J. DiGeorge syndrome: Consider the diagnosis. BMJ Case Rep 2022;15(2):e245164. DOI: 10.1136/bcr-2021-245164.
144. Pavone P, Pratico AD, Vitaliti G, et al. Hydranencephaly: Cerebral spinal fluid instead of cerebral mantles. Ital J Pediatr 2014;40:79. DOI: 10.1186/s13052-014-0079-1.
145. Pokhraj PS, Jigar JP, Chetan M, et al. Congenital porencephaly in a new born child. J Clin Diagn Res 2014;8(11):RJ01–RJ02. DOI: 10.7860/JCDR/2014/9981.5140.
146. Donn SM, Bowerman RA. Neonatal posthemorrhagic porencephaly: Ultrasonographic features. Am J Dis Child 1982;136(8):707–709. DOI: 10.1001/archpedi.1982.03970440051014.
147. Tinker SC, Gilboa SM, Moore CA, et al. Specific birth defects in pregnancies of women with diabetes: National birth defects prevention study, 1997-2011. Am J Obstet Gynecol 2020;222(2):176 e1–e11. DOI: 10.1016/j.ajog.2019.08.028.
148. Johnson CY, Rasmussen SA. Non-genetic risk factors for holoprosencephaly. Am J Med Genet C Semin Med Genet 2010;154C(1):73–85. DOI: 10.1002/ajmg.c.30242.
149. Solomon BD, Rosenbaum KN, Meck JM, et al. Holoprosencephaly due to numeric chromosome abnormalities. Am J Med Genet C Semin Med Genet 2010;154C(1):146–148. DOI: 10.1002/ajmg.c.30232.
150. Kruszka P, Martinez AF, Muenke M. Molecular testing in holoprosencephaly. Am J Med Genet C Semin Med Genet 2018;178(2):187–193. DOI: 10.1002/ajmg.c.31617.
151. Posey JE. Genome sequencing and implications for rare disorders. Orphanet J Rare Dis 2019;14(1):153. DOI: 10.1186/s13023-019-1127-0.
152. Barr M Jr, Cohen MM Jr. Holoprosencephaly survival and performance. Am J Med Genet 1999;89(2):116–120. PMID: 10559767.
153. Weiss K, Kruszka P, Guillen Sacoto MJ, et al. In-depth investigations of adolescents and adults with holoprosencephaly identify unique characteristics. Genet Med 2018;20(1):14–23. DOI: 10.1038/gim.2017.68.
154. Hadley DW, Kruszka P, Muenke M. Challenging issues arising in counseling families experiencing holoprosencephaly. Am J Med Genet C Semin Med Genet 2018;178(2):238–245. DOI: 10.1002/ajmg.c.31627.

CASE REPORT

Many Term infants with Persistent Patency of the Ductus Arteriosus could be Trisomy 21 Mosaics

Akhil Maheshwari[1,2], Srijan Singh[2,3], Varun Sharma[4], Papagudi G Subramanian[5], Amita S Garg[6,7,8]

Received on: 24 February 2024; *Accepted on*: 18 March 2024; *Published on*: 26 March 2024

Abstract

We report findings from a term infant with persistent patency of the ductus arteriosus (PDA). His fetal tests had shown some ambiguity for trisomy 21. However, he did not show any of the frequently-seen phenotypic features associated with trisomy 21 *in utero* or after birth, and the postnatal karyotype was reported as normal. One of our team members decided to request for a repeat karyotype and he was then identified as a mosaic for this aneuploidy. These observations are potentially important because the proportion of affected cells could very well be a determinant of the phenotypic variability seen in infants with Down syndrome. Hence, mosaicism might need to be meticulously excluded in patients who are presented with only one or more phenotypic features associated with trisomy 21. In this report, we have briefly reviewed the need for evaluation in such infants; the diagnosis requires specific evaluation of *in-vitro* cultured blood lymphocytes from the patients, siblings, and parents for somatic and germinal trisomy 21 mosaicism. The mechanisms underlying the origin of trisomy 21 mosaicism are still unclear; embryonic meiotic errors such as nondisjunction and anaphase lag, and subsequent mitotic malsegregation may be responsible. Uniparental disomy needs investigation. In the absence of somatic recombination, postzygotic malsegregation in an originally unaffected, disomy 21 zygote could also be a cause. The incidence of this condition in the community might be higher than hitherto believed.

Keywords: Anaphase lag, Case report, Copy-number alteration, Fluorescence *in situ* hybridization, Germinal trisomy 21 mosaicism, High-grade mosaics, Infant, Meiotic errors, Mitotic malsegregation, Newborn, Neonate, Nondisjunction, Postzygotic malsegregation, Somatic trisomy 21 mosaicism.

Newborn (2024): 10.5005/jp-journals-11002-0090

Highlights

- Infants with trisomy 21 frequently have persistent patency of ductus arteriosus (PDA) and/or other cardiac defects.
- We present a term male infant who had a persistent PDA. He did not show the phenotypic features that are usually seen in infants with trisomy 21 and initial tests showed a normal karyotype. Repeat tests identified him as a mosaic.
- The diagnosis was confirmed using examination of *in vitro* cultured blood lymphocytes for somatic and germinal trisomy 21 mosaicism.
- This disorder might be more common in the general population than previously believed, and hence, appropriate investigations might have to be considered in more patients.

In this brief report, we present findings from a term infant with persistent PDA. He did not show the characteristic phenotypic features of trisomy 21 but turned out to have mosaicism for this karyotypic aneuploidy. The incidence of mosaicism could be higher than previously believed, and could very well be an important mechanism of the phenotypic variability seen in this condition.[1–5] Hence, mosaicism might need to be meticulously excluded in patients with a limited or an overall less-severe phenotype.[5,6] Other than specific mutations in key genes, mosaicism could also be an important reason for the geographical variation in the cardiac manifestations of trisomy 21.[7–13] The severity of somatic manifestations, the incidence of complications, and the overall prognosis may also be related to mosaicism.[14–21]

[1]Department of Pediatrics, Louisiana State University, Shreveport, Louisiana, United States of America

[2]Global Newborn Society, Clarksville, Maryland, United States of America

[3]Department of Neonatology, Kailash Hospital, Noida, Uttar Pradesh, India

[4]Scientist, NMC Genetics, NMC Healthcare, Gurugram, Haryana, India

[5]Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Center, Homi Bhabha National Institute-University, Mumbai, Maharashtra, India

[6]Department of Non-invasive Cardiology, Fortis Hospital, New Delhi, India

[7]Down Syndrome Parents Society, New Delhi, India

[8]Down Syndrome Federation of India, Chennai, Tamil Nadu, India

Corresponding Author: Amita S Garg, Department of Non-invasive Cardiology, Fortis Hospital, New Delhi, India; Down Syndrome Parents Society, New Delhi, India; Down Syndrome Federation of India, Chennai, Tamil Nadu, India, Phone: +91 9312239463, e-mail: singhal.amita@gmail.com

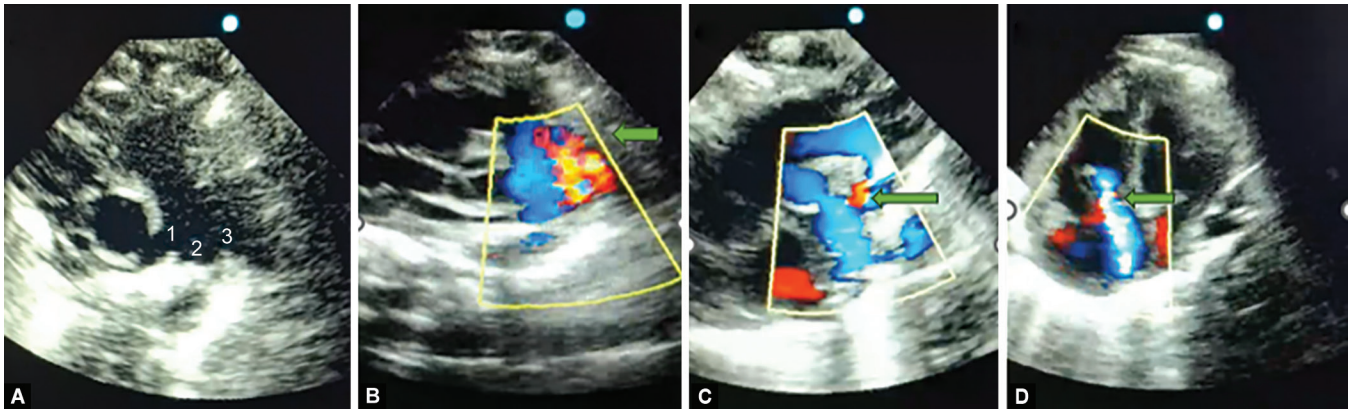
How to cite this article: Maheshwari A, Singh S, Sharma S, *et al*. Many Term infants with Persistent Patency of the Ductus Arteriosus could be Trisomy 21 Mosaics. Newborn 2024;3(1):61–64.

Source of support: Nil.

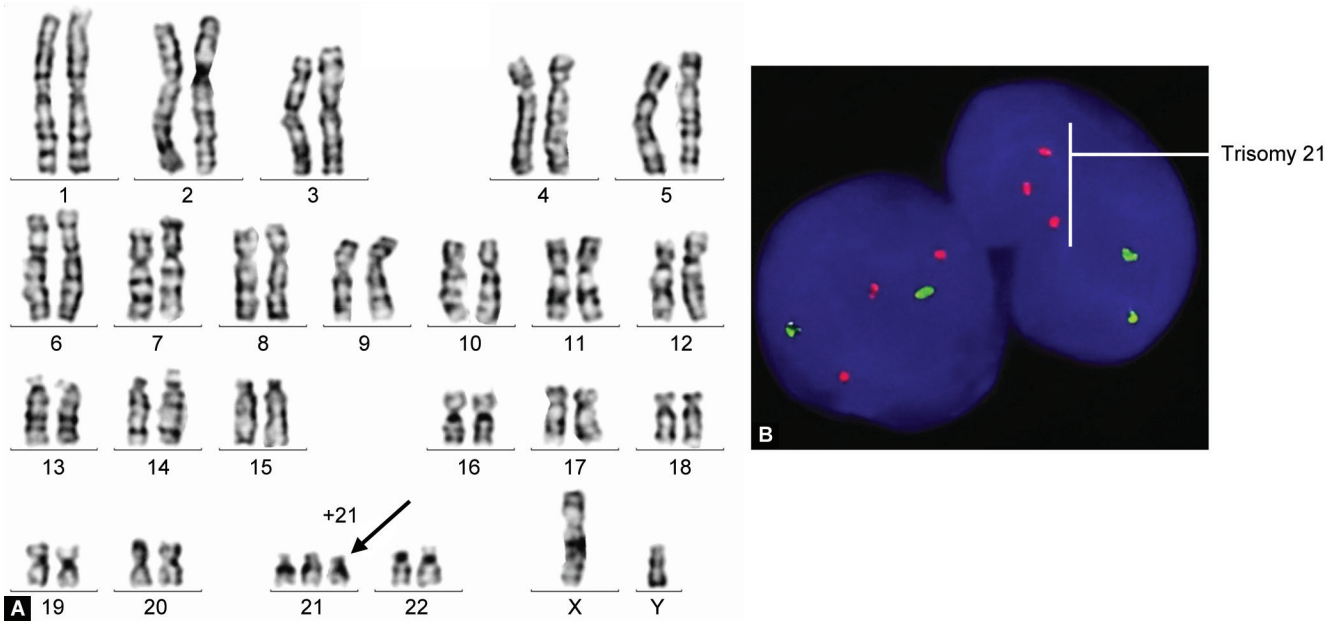
Conflict of interest: Dr Akhil Maheshwari is associated as Editor-in-Chief of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of the Editor-in-Chief and his research group.

Case Description

We evaluated plasma samples from a mother at 18 weeks' gestation for cell-free fetal DNA; there was a possibility of fetal aneuploidy in some samples but overall, the results were ambiguous. After delivery, the full-term male infant was noted to have a systolic murmur that was audible even on postnatal day 3. He did not show any of the frequently-seen phenotypic features associated with trisomy 21 on a detailed, careful physical examination performed by multiple care-providers, and the postnatal karyotype was reported



Figs 1A to D: Patent ductus arteriosus (PDA) in a full-term infant. (A) A ductal view showing the classical "three-legged stool appearance" of the PDA. The numerical labels in this photograph indicate (1) the right pulmonary artery, (2) the left pulmonary artery, and (3) the PDA; (B and C) PDA (red) at its junction with the aorta, in subcostal (B) and suprasternal (C) views; (D) A 4-chamber view showing a tricuspid regurgitation jet (blue) indicating pulmonary hypertension related to ductal patency



Figs 2A and B: (A): Noninvasive prenatal testing was performed to detect fetal aneuploidy in cell-free DNA in maternal plasma. Samples were analyzed on an Affymetrix microarray platform by parallel shotgun sequencing. Sequencing libraries were prepared from plasma DNA and sequenced to generate millions of short-sequence reads. These were mapped to the reference human genome, allowing determination of the chromosome of origin for each sequence. The number of mapped sequences originating from each chromosome was calculated and normalized. Aneuploidy was noted by comparing test samples to known normal reference samples, using a z-score approach; This figure shows a karyotype showing trisomy 21 due to nondisjunction of chromosome 21 in affected cells. This extra copy of chromosome 21 gives a total chromosome count of 47. Most mosaics show an abnormal karyotype in only 1–2 of the usual 10–15 tested cells. In India, we are also seeing a larger number of infants with Robertsonian translocation t(14q;21q); not shown; (B) Fluorescence *in-situ* hybridization (FISH) can detect trisomy 21. Lymphocytes isolated from a peripheral blood (or bone marrow) sample and were cultured *ex-vivo*, and then smeared on glass slides. The slides were aged and then treated with pepsin to expose the nuclear chromatin in metaphase chromosomes. Fluorescent-labeled locus-specific DNA probes were used to target the long arm of chromosome 21 (21q22.13-q22.2 and 21q22.2-q22.3 regions). Before hybridization, the probes were heat-denatured to allow access to complementary chromosomal DNA. The denatured probes were applied to the slide and hybridization carried out at specific binding sites by overnight incubation at 37°C. Unbound probes were removed by washing and the nuclei were counterstained with DAPI. Fluorescence microscopy was used to visualize the labeled chromosomes 21. Unlike normal cells that show two homologs of chromosome 21, those with trisomy 21 show 3. A minimum of 10 metaphase spreads were examined and trisomy 21 was reported if ≥3 signals were seen in ≥10% cells; This figure shows trisomy 21 in FISH analysis of interphase cells. The cells were hybridized with an orange chromosome 21-specific probe and a control green probe for chromosome 12. Cells with trisomy 21 showed 3 orange signals instead of the normal 2. Infants who are mosaics for trisomy 21 have some cells with 2 and others 3 due to postzygotic nondisjunction. Magnification: 400x

as normal. An echocardiogram on postnatal day 4 showed a patent "ductus arteriosus" with a significant left-to-right shunt (Fig. 1). Signs of congestive heart failure became evident at 4–5 weeks, and so the duct was surgically ligated at 6 weeks of postnatal age. The recovery was uneventful; the infant was discharged from the hospital 1 week later. As is evident in the above description, this clinical course was typical of an infant with a hemodynamically-significant PDA. However, in view of the prenatal ambiguity in the karyotype, one of our team members decided to request another evaluation of the karyotype at 2 weeks after birth; he was identified as a mosaic for trisomy 21 (Fig. 2).[22]

Brief Review

Current information suggests that 2–4% of all infants with trisomy 21 are karyotypic mosaics.[7,23] We know that nearly 95% of all cases of Down syndrome result from chromosomal nondisjunction of chromosome 21 (47, XX, +21 or 47, XY, +21) at conception.[2,24,25] The process of oogenesis is lengthy and involves meiotic arrest, which makes the ova more vulnerable to malsegregation of chromosomes than spermatogenesis.[26,27] Increasing age of the ova can also promote the degradation of cellular proteins involved in spindle formation,[28,29] sister chromatid cohesion,[30] and anaphase separation of sister chromatids in oocytes, which increases the risk of nondisjunction during meiosis.[31] Such translocations are often familial, most frequently involving chromosomes 14 and 21.[7] In 1% of all cases of Down syndrome, the extra chromosome 21 material originates from other rearrangements.[32]

The etiopathogenesis of mosaicism is still unclear. Unlike infants with "complete" trisomy 21 who show the extra chromosome 21 in all cells, most mosaics show an abnormal karyotype in only 1–2 of the usual 10–15 tested cells (Fig. 1). In these "possibly positive" patients, the analysis is usually extended to 50 or more cells. The diagnosis of mosaicism may require examination of *in vitro* cultured blood lymphocytes from the patients, siblings, and parents for somatic and germinal trisomy 21 mosaicism.[33,34] Currently, most mosaics who are being identified are possibly the ones with the "high-grade"condition[35,36] but the sensitivity is likely to improve with advancement in methods of testing and as our threshold for requesting these tests improves.[36,37]

The chromosomal abnormalities in mosaics do not appear to be unique.[38] Embryonic meiotic errors such as nondisjunction and anaphase lag, and subsequent mitotic malsegregation may be responsible just as in the majority of infants with Down syndrome.[39] Uniparental disomy needs investigation.[40] In the absence of somatic recombination, mosaicism could originate from postzygotic malsegregation in an originally unaffected, disomy 21 zygote.[36] Postfertilization formation of a Robertsonian translocation t(14q;21q) and an isochromosome is also now being recognized with increasing frequency.[41–43]

Currently, the methodology of choice for detecting the number of chromosomes 21 is fluorescence *in situ* hybridization (FISH; Fig. 2).[44–46] The sensitivity of these tests in conventional cytogenetic analysis can be enhanced by testing cultured blood lymphocytes; these tests may show trisomy 21 in 1 in 60 metaphase cells.[36] When combined with FISH analyses of interphase nuclei from uncultured blood cells, trisomy 21 may be seen in up to 10% cells.[36,47] Similarly, trisomy 21 can also be identified in cells obtained in chorionic villus sampling/amniocentesis.[48]

We also need efforts to identify trisomy 21 mosaics who might have alterations in systems other than the hematopoietic system.[49] Evaluation of mucosal cells obtained from buccal swabs, cultured skin fibroblasts, or even DNA from whole-skin biopsies can be helpful.[50] Copy-number alteration can be identified with sensitivity rates as high as 20% using next-generation sequencing (NGS) of whole-skin biopsy DNA.[51–54]

Authors' Contributions

Authors AM, SS, PGS, and ASG provided data and wrote the manuscript; VS reviewed and made important and critical revisions.

Orcid

Akhil Maheshwari https://orcid.org/0000-0003-3613-4054
Srijan Singh https://orcid.org/0000-0002-2103-5232
Papagudi G Subramanian https://orcid.org/0000-0001-9107-5937

References

1. Hulten MA, Jonasson J, Iwarsson E, et al. Trisomy 21 mosaicism: We may all have a touch of Down syndrome. Cytogenet Genome Res 2013;139(3):189–192. DOI: 10.1159/000346028.
2. Patterson D, Costa AC. Down syndrome and genetics: A case of linked histories. Nat Rev Genet. 2005;6(2):137–147. DOI: 10.1038/nrg1525.
3. Zhang Y, Zhong M, Zheng D. Chromosomal mosaicism detected by karyotyping and chromosomal microarray analysis in prenatal diagnosis. J Cell Mol Med 2021;25(1):358–366. DOI: 10.1111/jcmm.16080.
4. Ko JM. Genetic syndromes associated with congenital heart disease. Korean Circ J 2015;45(5):357–361. DOI: 10.4070/kcj.2015.45.5.357.
5. Martinez–Glez V, Tenorio J, Nevado J, et al. A six-attribute classification of genetic mosaicism. Genet Med 2020;22(11):1743–1757. DOI: 10.1038/s41436-020-0877-3.
6. Leon E, Zou YS, Milunsky JM. Mosaic Down syndrome in a patient with low-level mosaicism detected by microarray. Am J Med Genet A 2010;152A(12):3154–3156. DOI: 10.1002/ajmg.a.33739.
7. Plaiasu V. Down syndrome: Genetics and cardiogenetics. Maedica (Bucur) 2017;12(3):208–213. PMID: 29218069.
8. Jacobs EG, Leung MP, Karlberg J. Distribution of symptomatic congenital heart disease in Hong Kong. Pediatr Cardiol 2000;21(2):148–157. DOI: 10.1007/s002469910025.
9. Lo NS, Leung PM, Lau KC, et al. Congenital cardiovascular malformations in Chinese children with Down's syndrome. Chin Med J (Engl) 1989;102(5):382–386. PMID: 2530065.
10. Kim MA, Lee YS, Yee NH, et al. Prevalence of congenital heart defects associated with Down syndrome in Korea. J Korean Med Sci 2014;29(11):1544–1549. DOI: 10.3346/jkms.2014.29.11.1544.
11. de Rubens Figueroa J, del Pozzo Magaña B, Hach JLP, et al. Rev Esp Cardiol 2003;56(9):894–899. DOI: 10.1016/s0300-8932(03)76978-4.
12. Vida VL, Barnoya J, Larrazabal LA, et al. Congenital cardiac disease in children with Down's syndrome in Guatemala. Cardiol Young 2005;15(3):286–290. DOI: 10.1017/S1047951105000582.
13. Elmagrpy Z, Rayani A, Shah A, et al. Down syndrome and congenital heart disease: Why the regional difference as observed in the Libyan experience? Cardiovasc J Afr 2011;22(6):306–309. DOI: 10.5830/CVJA-2010-072.
14. Baird PA, Sadovnick AD. Life expectancy in Down syndrome. J Pediatr 1987;110(6):849–854. DOI: 10.1016/s0022-3476(87)80395-5.
15. Baird PA, Sadovnick AD. Life tables for Down syndrome. Hum Genet 1989;82(3):291–292. DOI: 10.1007/BF00291175.
16. Esbensen AJ. Health conditions associated with aging and end of life of adults with Down syndrome. Int Rev Res Ment Retard 2010;39(C):107–126. DOI: 10.1016/S0074-7750(10)39004-5.

17. Ali FE, Al-Bustan MA, Al-Busairi WA, et al. Cervical spine abnormalities associated with Down syndrome. Int Orthop 2006;30(4):284–289. DOI: 10.1007/s00264-005-0070-y.
18. Xavier AC, Taub JW. Acute leukemia in children with Down syndrome. Haematologica 2010;95(7):1043–1045. DOI: 10.3324/haematol.2010.024968.
19. Kim GE, Sin DS, Kim SS, et al. End-stage renal disease in a Down syndrome patient caused by delayed diagnosis of nonneurogenic bladder: A case report. Medicine (Baltimore) 2019;98(15):e15145. DOI: 10.1097/MD.0000000000015145.
20. Danopoulos S, Deutsch GH, Dumortier C, et al. Lung disease manifestations in Down syndrome. Am J Physiol Lung Cell Mol Physiol 2021;321(5):L892–L899. DOI: 10.1152/ajplung.00434.2020.
21. Dierssen M. Down syndrome: The brain in trisomic mode. Nat Rev Neurosci 2012;13(12):844–858. DOI: 10.1038/nrn3314.
22. Truty R, Rojahn S, Ouyang K, et al. Patterns of mosaicism for sequence and copy-number variants discovered through clinical deep sequencing of disease-related genes in one million individuals. Am J Hum Genet 2023;110(4):551–564. DOI: 10.1016/j.ajhg.2023.02.013.
23. Thorpe J, Osei–Owusu IA, Avigdor BE, et al. Mosaicism in human health and disease. Annu Rev Genet 2020;54:487–510. DOI: 10.1146/annurev-genet-041720-093403.
24. Ghosh S, Feingold E, Dey SK. Etiology of Down syndrome: Evidence for consistent association among altered meiotic recombination, nondisjunction, and maternal age across populations. Am J Med Genet A 2009;149A(7):1415–1420. DOI: 10.1002/ajmg.a.32932.
25. Day T, Taylor PD. Chromosomal drive and the evolution of meiotic nondisjunction and trisomy in humans. Proc Natl Acad Sci USA 1998;95(5):2361–2365. DOI: 10.1073/pnas.95.5.2361.
26. Oliver TR, Feingold E, Yu K, et al. New insights into human nondisjunction of chromosome 21 in oocytes. PLoS Genet 2008;4(3):e1000033. DOI: 10.1371/journal.pgen.1000033.
27. DiLuigi A, Weitzman VN, Pace MC, et al. Meiotic arrest in human oocytes is maintained by a Gs signaling pathway. Biol Reprod 2008;78(4):667–672. DOI: 10.1095/biolreprod.107.066019.
28. Hawley RS, Frazier JA, Rasooly R. Separation anxiety: The etiology of nondisjunction in flies and people. Hum Mol Genet 1994;3(9):1521–1528. DOI: 10.1093/hmg/3.9.1521.
29. Goutas A, Outskouni Z, Papathanasiou I, et al. The establishment of mitotic errors-driven senescence depends on autophagy. Redox Biol 2023;62:102701. DOI: 10.1016/j.redox.2023.102701.
30. Wolstenholme J, Angell RR. Maternal age and trisomy: A unifying mechanism of formation. Chromosoma 2000;109(7):435–438. DOI: 10.1007/s004120000088.
31. MacLennan M, Crichton JH, Playfoot CJ, et al. Oocyte development, meiosis and aneuploidy. Semin Cell Dev Biol 2015;45:68–76. DOI: 10.1016/j.semcdb.2015.10.005.
32. Pelleri MC, Locatelli C, Mattina T, et al. Partial trisomy 21 with or without highly restricted Down syndrome critical region (HR-DSCR): Report of two new cases and reanalysis of the genotype-phenotype association. BMC Med Genomics 2022;15(1):266. DOI: 10.1186/s12920-022-01422-6.
33. Papavassiliou P, Charalsawadi C, Rafferty K, et al. Mosaicism for trisomy 21: A review. Am J Med Genet A 2015;167A(1):26–39. DOI: 10.1002/ajmg.a.36861.
34. Papavassiliou P, York TP, Gursoy N, et al. The phenotype of persons having mosaicism for trisomy 21/Down syndrome reflects the percentage of trisomic cells present in different tissues. Am J Med Genet A 2009;149A(4):573–583. DOI: 10.1002/ajmg.a.32729.
35. Sanchez–Pavon E, Mendoza H, Garcia–Ferreyra J. Trisomy 21 and assisted reproductive technologies: A review. JBRA Assist Reprod 2022;26(1):129–1241. DOI: 10.5935/1518-0557.20210047.
36. Hulten MA, Jonasson J, Nordgren A, et al. Germinal and somatic trisomy 21 mosaicism: How common is it, what are the implications for individual carriers and how does it come about? Curr Genomics 2010;11(6):409–419. DOI: 10.2174/138920210793176056.
37. Munne S, Wells D. Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing. Fertil Steril 2017;107(5):1085–1091. DOI: 10.1016/j.fertnstert.2017.03.024.
38. Taylor TH, Gitlin SA, Patrick JL, et al. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. Hum Reprod Update 2014;20(4):571–581. DOI: 10.1093/humupd/dmu016.
39. Pangalos C, Avramopoulos D, Blouin JL, et al. Understanding the mechanism(s) of mosaic trisomy 21 by using DNA polymorphism analysis. Am J Hum Genet 1994;54(3):473–4781. PMID: 8116616.
40. Chen CP, Liou JD, Chern SR, et al. Prenatal diagnosis of maternal uniparental disomy 21 in association with low-level mosaic trisomy 21 at amniocentesis in a pregnancy associated with intrauterine growth restriction and a favorable outcome. Taiwan J Obstet Gynecol 2022;61(1):146–149. DOI: 10.1016/j.tjog.2021.11.025.
41. Bandyopadhyay R, McCaskill C, Knox–Du Bois C, et al. Mosaicism in a patient with Down syndrome reveals post-fertilization formation of a Robertsonian translocation and isochromosome. Am J Med Genet A 2003;116A(2):159–163. DOI: 10.1002/ajmg.a.10113.
42. Petersen MB, Adelsberger PA, Schinzel AA, et al. Down syndrome due to de novo Robertsonian translocation t(14q;21q): DNA polymorphism analysis suggests that the origin of the extra 21q is maternal. Am J Hum Genet 1991;49(3):529–536. PMID: 1831959.
43. Shaffer LG, Agan N, Goldberg JD, et al. American College of Medical Genetics statement of diagnostic testing for uniparental disomy. Genet Med 2001;3(3):206–211. DOI: 10.1097/00125817-200105000-00011.
44. Iourov IY, Vorsanova SG, Yurov YB. Chromosomal mosaicism goes global. Mol Cytogenet 2008;1:26. DOI: 10.1186/1755-8166-1-26.
45. Namba A, Nishiyama M, Weiser JJ, et al. Prenatal diagnosis of complex rearrangement of chromosome 21: The significance of interphase and metaphase fluorescence in situ hybridization and comparative genomic hybridization. Clin Case Rep 2013;1(2):50–53. DOI: 10.1002/ccr3.22.
46. Witters I, Devriendt K, Legius E, et al. Rapid prenatal diagnosis of trisomy 21 in 5049 consecutive uncultured amniotic fluid samples by fluorescence in situ hybridisation (FISH). Prenat Diagn 2002;22(1):29–33. DOI: 10.1002/pd.225.
47. Davies AF, Barber L, Murer–Orlando M, et al. FISH detection of trisomy 21 in interphase by the simultaneous use of two differentially labelled cosmid contigs. J Med Genet 1994;31(9):679–685. DOI: 10.1136/jmg.31.9.679.
48. Beverstock GC, Hansson K, Helderman–van den Enden AT, et al. A near false-negative finding of mosaic trisomy 21: A cautionary tale. Prenat Diagn 1998;18(7):742–746. PMID: 9706658.
49. Li J, Kalev–Zylinska ML. Advances in molecular characterization of myeloid proliferations associated with Down syndrome. Front Genet 2022;13:891214. DOI: 10.3389/fgene.2022.891214.
50. Livy A, Lye S, Jagdish CK, et al. Evaluation of quality of DNA extracted from buccal swabs for microarray based genotyping. Indian J Clin Biochem 2012;27(1):28–33. DOI: 10.1007/s12291-011-0154-y.
51. Singh AK, Olsen MF, Lavik LAS et al. Detecting copy-number variation in next generation sequencing data from diagnostic gene panels. BMC Med Genomics 2021;14(1):214. DOI: 10.1186/s12920-021-01059-x.
52. Qin D. Next-generation sequencing and its clinical application. Cancer Biol Med 2019;16(1):4–10. DOI: 10.20892/j.issn.2095-3941.2018.0055.
53. Ramachandran D, Mulle JG, Locke AE, et al. Contribution of copy-number variation to Down syndrome-associated atrioventricular septal defects. Genet Med 2015;17(7):554–560. DOI: 10.1038/gim.2014.144.
54. Rambo–Martin BL, Mulle JG, Cutler DJ, et al. Analysis of copy-number variants on chromosome 21 in Down syndrome-associated congenital heart defects. G3 (Bethesda) 2018;8(1):105–111. DOI: 10.1534/g3.117.300366.