October–December 2022 Volume 1 Issue 4 newborn Official Journal of the Global Newborn Society ISSN: 2769-514X

newborn

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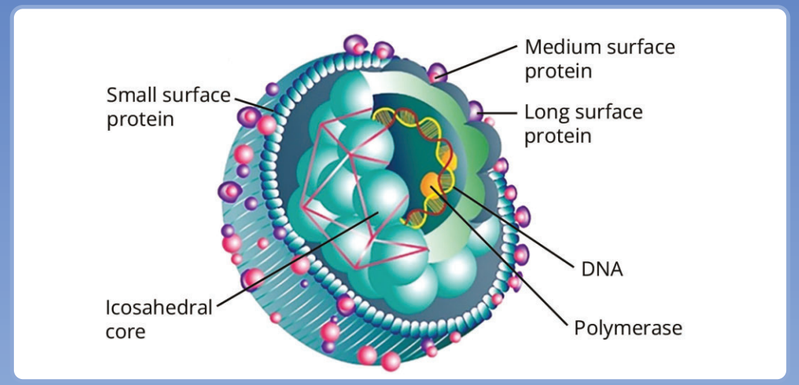
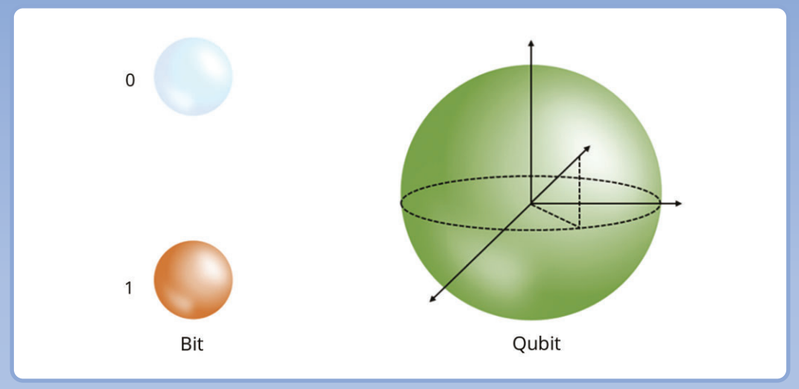
Volume 1
Issue 4
ISSN: 2769-514X



Hepatitis B Infections in Neonates



Quantum Cryptography for Securing Personal Health Information in Hospitals

Other highlights:

Establishment of the First Religiously-Compliant Human Milk Bank in Bangladesh

Fats in Human Milk: 2022 Updates on Chemical Composition

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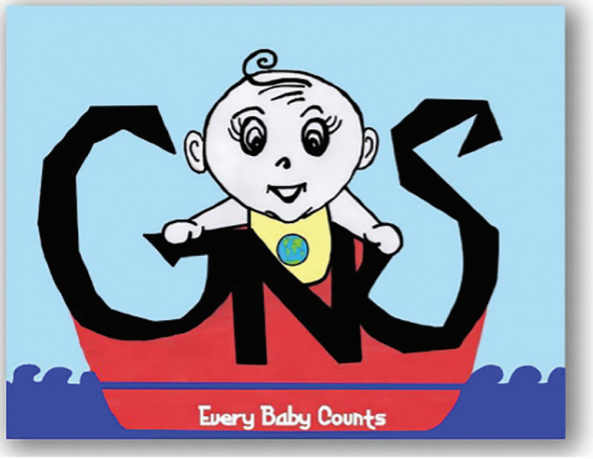

Global Newborn Society

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

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

Our logo above, a hand-drawn painting, graphically summarizes our thought-process. There is a lovable little young infant exuding innocent, genuine happiness. The curly hair, shape of the eyes, long 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 the hope of peace and tranquility is not completely lost yet. The acronym letters, GNS, on the starboard are made of casted metal and are pillars of strength. However, the angular rough edges need continued polishing to ascertain adequacy and progress. The red color of the boat symbolizes our affection. The expression "*Every Baby Counts*" seen on the boat's draft below the waterline indicates our commitment to philanthropy, and if needed, to altruism that does not always need to be visible. The shadow behind the picture shows that it has been glued on a solid wall, one built out of our adoption and commitment.

Design of the Journal Cover

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

Instructions to Authors

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

Issue Information

Volume 1, Issue 4; October-December 2022
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, Darya Ganj, Delhi - 110 002
Phone: +91 11 4357 4357, Fax: +91 11 4357 4314

Contents

EDITORIAL

10.5005/newborn-1-4-iv

Understanding the Impact of Maternal Health on Neonatal Disease: A New Horizon

Critically ill neonates, whether premature or those born closer to full-term gestation, can have adverse outcomes following untimely exposure to many physiological and other potentially pathogenic stimuli *in utero* and after birth.[1–3] In many organ systems, even minor alterations in the course of maturation can cause abnormalities.[4,5] Carefully designed studies are needed to extrapolate the experience from one part of the world to another;[6,7] there are important genetic, infectious, and cultural differences that might affect outcomes. However, in many situations, there are vertical influences where information about maternal health can help us in understanding neonatal disease and in predicting its outcomes.[8]

We have long believed that the fetus grows in a sterile uterovaginal tract.[9] However, recent studies have identified bacterial flora in the birth canal.[10] The impact of this colonization on a fetus developing *in utero* is still unclear, but there is a possibility that the presence of these bacteria on mucosal surfaces might alter perinatal outcomes.[11] The effect on the developing fetal immune system also needs study, particularly with emerging information about many subclinical infections prevalent in many parts of the world.[12,13] There is a need for improved understanding of the impact of these infections on the developing immune system.

In addition to the bacterial flora, nutrition is another important stimulant for the rapidly growing organs, be it the central nervous system, immunity, or the endocrine system.[14] The nutritional experiences of the newborn infants may have lifelong effects.[15] Human milk contains fats in high concentrations, which seem to be important as a source of energy and in maturation of the neurological and mucosal immune systems.[16,17] Milk fat content and composition are highly dynamic parameters in early infancy, and need careful analysis.[18] However, despite increasing awareness about the importance of human milk, we also now know about the factors that may curtail its availability. Mothers of infants who are critically ill experience a high degree of anxiety, and may not always able to provide milk for their infants.[19,20] The availability of human milk may also be limited in situations such as maternal illness, ongoing drug therapy, or substance abuse.[21] In these situations, human milk banks can be an important resource but there are many restrictions—natural and human-created.[22]

We have been able to make important progress in the treatment of many life-threatening neonatal illnesses.[23] These advances have not only reduced neonatal deaths, but have also facilitated major societal changes; fewer neonatal deaths have helped in reducing the total number of pregnancies per woman as she is reassured about the number of surviving infants. Fewer pregnancies have helped reduce maternal mortality.[24,25]

In the *Newborn*, we aim to cover problems that a baby might develop *in utero*, the perinatal period, following birth, and the implications of these abnormalities during the first 1000 days after birth. In this 4th issue of the first volume, we present 8 important articles (**Fig. 1**). There are 3 important papers focused on the impact of infectious agents on the developing immunity. Padhi *et al*.[26] have examined the prevalence of gram-negative bacteria in maternal cervical secretions. They are investigating early-onset sepsis (EOS) seen within 72 hours following birth.[2,27] These infants likely acquire the bacterial pathogens from the maternal cervical/vaginal secretions during the perinatal period.[27–29] In the West, EOS is caused most frequently by gram-positive bacteria such as group B Streptococci (GBS), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus viridans*, and *Enterococcus* spp. Gram-negative pathogens are seen in some cases.[27,30–33] However, in tropical and peri-equatorial regions, EOS may be caused more frequently due to gram-negative pathogens.[34–38]These authors evaluated the literature for the quality of data, and performed a systematic review and meta-analysis of 15 studies. They show that gram-negative colonization of the maternal cervical-vaginal tract in tropical/peri-equatorial regions of the world was more frequent than previously recognized. Early identification of these pathogens may help in timely evaluation and treatment of these infants.

In another study, Anand *et al*.[39] reviewed congenital/perinatal hepatitis B infections in neonates. Hepatitis B infections are estimated to affect more than 2 billion people worldwide. The overall prevalence of HBsAg positivity in plasma may be only 3.5%, but it varies depending upon the geographic area. In exposed infants, universal hepatitis B vaccination and the administration of hepatitis B

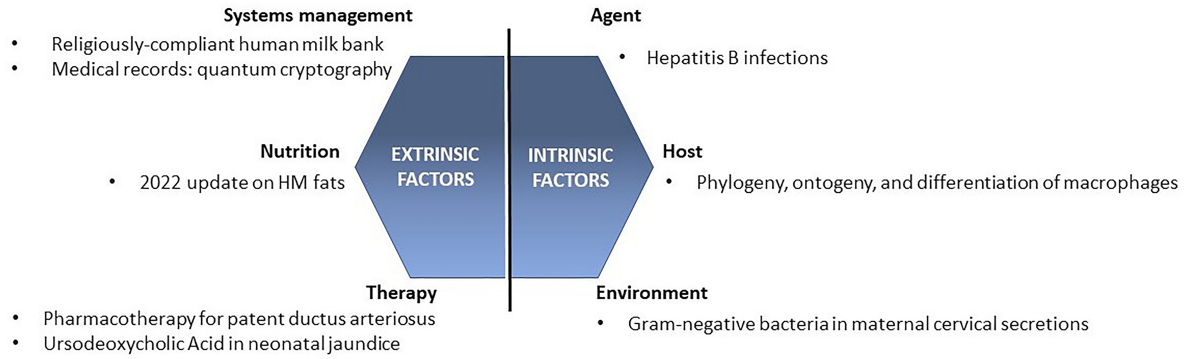

Fig. 1: **Areas of focus in the *Newborn*, volume 1, issue 4.** The *newborn* has expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three extrinsic factors originate in therapy, nutrition, and systems management.

immunoglobulin (HBIg) within 12 hours following birth can reduce the risk of perinatal infection.[40] Knowing the importance of these fetal/neonatal infections, we also needed to study the host defense mechanisms. Unlike in adults, macrophages are the major resource for innate cellular immunity in neonates.[41] In terms of the numerical content, the largest macrophage population is in the gastrointestinal tract.[42] An article has been devoted to track the ontogeny, phylogeny, and the development of intestinal macrophages.[43]

In a survey of the readership of the *Newborn*, we noted that the readers sought a comprehensive review of fats in human milk. The fat content of human milk is high, and hence, these constitute an important set of nutrients for newborn infants. Fats are also needed for neurological development in newborn infants. Many readers suggested that the Global Newborn Society needs to assume responsibility here and regularly provide information on milk fats. Hence, this article has been named as providing the 2022 updates on chemical composition of human milk.[44]

This issue contains a very important article that traces the development of a religiously compliant human milk bank (HMB) in Bangladesh. Rahman et al.[45] have made major efforts to develop and maintain this facility. HMBs similar to those in the West have been difficult to establish in Muslim countries as the Islamic law does not allow consumption of unidentified donated milk from multiple donors. Human milk is known to be important for nutrition in premature and critically ill infants, and so there is a need to develop religiously compliant and conditionally identified HMBs in Muslim countries. In these milk banks, every mother's milk is processed and stored separately, and the milk provided by one mother can be provided to an infant from a different family only after appropriately counseling both families about the Islamic law of prohibition of future marriages between milk siblings. Documents related to these issues are provided to both families and data need to be maintained for future reference.

There are two important papers on evaluation of the quality of evidence for treatment modalities in neonatal intensive care units. Arif et al.[46] performed a systematic review to evaluate the effect of ursodeoxycholic acid in unconjugated hyperbilirubinemia in term neonates treated with phototherapy. The combination of these two treatments reduces the bilirubin levels and duration of phototherapy, although there is a need for further treatment. In another article, Srivastava et al.[47] evaluated pharmacologic therapy for patent ductus arteriosus closure[7] in preterm small-for-gestational-age infants. They observed a similar rate of PDA closure following the first course of non-steroidal anti-inflammatory drug (NSAID) therapy between appropriately grown and growth-restricted neonates. However, severe growth restriction[48] (birth weight Z-score[49] below –2) was associated with higher rates of PDA ligation as compared to normally grown infants.

Finally, we have a review article describing the possibility of using quantum cryptography for securing personal health information in hospitals.[50] In our healthcare systems, we have been able to efficiently store healthcare information, retrieve it in a timely fashion, and ensure its safety.[51] However, the data are increasing rapidly and our current computational systems could well become inadequate in the not-so-distant future.[52] This article reviews the possibility of using quantum computing algorithms/devices that can provide elegant solutions based on subatomic interactions.[53,54]

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ORIGINAL RESEARCH

Efficacy of Pharmacologic Therapy for Patent Ductus Arteriosus Closure in Preterm Small for Gestational Age Infants

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Received on: 12 November 2022; *Accepted on:* 25 November 2022; *Published on:* 23 December 2022

Abstract

Objective: To determine the association between the degree of intrauterine growth restriction (IUGR) [defined by birth weight (BW) *Z*-score] and the efficacy of pharmacologic patent ductus arteriosus (PDA) closure and the rate of surgical PDA ligation in preterm neonates.

Materials and methods: In this retrospective cohort study, we included neonates born below 30 weeks' gestational age (GA), who received medical treatment for PDA between January 2010 and December 2018. Birth weight *Z*-scores were calculated using Olsen nomograms and classified into three categories: above −0.5; from −0.5 to −2.0; below−2. We compared responses to PDA treatment with non-steroidal anti-inflammatory drugs (NSAIDs) and PDA ligations between these groups utilizing multivariable logistic regression analysis.

Results: Of 769 neonates with PDA, 517 (67.2%) neonates received medical treatment for PDA. Of which, 323 (62.5%) had BW *Z*-score above −0.5, 154 (29.8%) had from −0.5 to −2.0., and 40 (7.7%) had below −2. The efficacy of the first course of NSAIDs for the PDA closure was not different among the three groups (51% vs 49% vs 50%). Multivariable logistic regression analysis showed there was no significant difference in PDA closure rate following the first course of NSAIDs between neonates with BW *Z*-score below −2 and those with BW *Z*-score above −0.5 [adjusted odds ratio (aOR): 0.68; 95% CI: 0.33–1.39] as well as those with BW *Z*-score from −0.5 to −2.0 (aOR: 0.89; 95% CI: 0.59–1.35). However, the odds of PDA ligation were significantly higher among neonates with BW *Z*-scores below −2 (aOR: 2.67, 95% CI: 1.12–6.34) but not among neonates with *Z*-scores from −0.5 to −2.0 (aOR: 1.41; 95% CI: 0.84–2.39), as compared to those with BW *Z*-scores above −0.5.

Conclusion: We observed a similar rate of PDA closure following the first course of NSAIDs between appropriately grown and growth-restricted neonates. However, severe growth restriction (BW *Z*-score below −2) is associated with higher rates of PDA ligation as compared to normally grown infants.

Keywords: Ibuprofen, Indomethacin, Intrauterine growth restriction, Patent ductus arteriosus, Prematurity.

Newborn (2022): 10.5005/jp-journals-11002-0048

Introduction

Patent ductus arteriosus is one of the most common cardiovascular conditions encountered in preterm neonates. Approximately 70% of neonates below 28 weeks GA and 80% of neonates born at 24–25 weeks GA are affected with PDA during their neonatal intensive care unit (NICU) stay.[1] A hemodynamically significant PDA (HsPDA) can lead to left-to-right shunting of blood and undesirable pulmonary, renal, and gastrointestinal effects, including pulmonary edema and hemorrhage, congestive cardiac failure, intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), feeding intolerance, poor weight gain, bronchopulmonary dysplasia (BPD) and death.[2]

Non-steroidal anti-inflammatory agents such as Indomethacin and ibuprofen are the two commonly used medications for PDA closure, whereas acetaminophen is an emerging potentially less toxic option with similar efficacy to the NSAIDs.[3] The PDA closure rates after an initial course of pharmacologic treatment vary from 48% to 98.5%.[4] In Canada, conservative PDA management in neonates below 32 weeks' gestation has increased from 14% to 38% from 2006 to the year 2012, whereas the surgical treatment rates have decreased from 7.1% to 2.5% during this epoch.[5]

Compared to appropriately grown preterm neonates, IUGR preterm neonates are at increased risk of neonatal mortality and morbidities.[6,7] In most cases, fetuses who show IUGR are delivered small for gestational age (SGA, BW less than tenth percentile for GA).[6] Growth-restricted preterm neonates not only have a higher incidence of significant PDA but also more frequent and earlier hemodynamic consequences of PDA.[8,9] Heymann reported a higher failure rate of indomethacin therapy for PDA closure among SGA neonates in 1987[10] and speculated that this could be due to altered levels of prostaglandins, or differences in receptor number or sensitivity. Few studies have examined the efficacy of pharmacologic PDA closure among neonates based on their

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How to cite this article: Srivastava A, Kaur S, Kamaluddeen M, *et al*. Efficacy of Pharmacologic Therapy for Patent Ductus Arteriosus Closure in Preterm Small for Gestational Age Infants. Newborn 2022;1(4): 327–332.

Source of support: Nil

Conflict of interest: None

degree of IUGR, with differing results.[11,12] In a single-center study from France, Madeleneau et al. reported the impact of SGA on the treatment for PDA in extremely preterm infants and concluded that failure of the first course of ibuprofen increased with the degree of growth restriction, reaching a maximum of 12.8-fold higher risk of failure, according to a gradient that intensified with regression adjustments.[11] A multicentre study from the United States reported that SGA infants with *Z*-scores between −2 and −0.5 were more likely to have PDA surgery following indomethacin and/or ibuprofen treatment compared to normally grown infants.[12] Infants with *Z*-scores below −2 had more than 3-fold increased odds of experiencing the composite of PDA surgery following pharmacologic treatment or death, and those with *Z*-scores between −2 and −0.5 had 1.5-fold increased odds.

We hypothesize that preterm growth-restricted infants are less likely to respond to medical treatment for PDA and may need surgical PDA ligation. The objective of this study was to determine the association between the degree of IUGR (defined by BW *Z*-score) and the efficacy of pharmacologic PDA closure and subsequent surgical PDA ligation rate in preterm neonates.

Materials and Methods

In this retrospective cohort study, we included neonates with GA below 30 weeks at birth admitted to the NICU at Foothills Medical Centre in Calgary, Alberta, Canada, between January 2010 and December 2018, who received pharmacological treatment (indomethacin, ibuprofen, or acetaminophen) for PDA. We excluded neonates with major congenital anomalies, neonates who received NSAIDs solely for the purpose of pain management or prophylactic indomethacin for prevention of brain injury, those who died before 48 hours of age or who had primary PDA ligation without any medical treatment. The Conjoint Health Research Ethics Board at the University of Calgary approved this study (REB19-1787).

In our NICU, the decision to treat a PDA was at the discretion of the attending physician and is based on clinical signs and symptoms (such as new onset murmur, wide pulse pressure, systemic hypotension, bounding pulses, and new onset changes in respiratory requirements considered to be due to PDA or clinical deterioration ascribed largely to the PDA), supplemented by echocardiographic confirmation of HsPDA. For this study, HsPDA was defined as a trans-ductal diameter measured at its narrowest dimension of above 1.5 mm, a peak systolic velocity below 1.5 m/second or left atrium (LA) to aortic (Ao) ratio (LA: Ao) above 1.5, as well as either predominantly or complete left-to-right shunting with or without absent or reversed diastolic flow in celiac or superior mesenteric artery.[13,4]

Indomethacin (Indocid P.D.A, Merck Frosst, Kirkland, Canada) is given intravenously in 3 doses at 12-hour intervals with doses varied by age (below 48 hours of life, 0.2, 0.1, and 0.1 mg/kg/dose; 2–7 days of life 0.2 mg/kg for each of the 3 doses; and above 7 days of life, 0.2, 0.25, and 0.25 mg/kg/dose). Ibuprofen is given in 3 doses of 10, 5, and 5 mg/kg/dose at 24-hour intervals. The second course of medical therapy is given for a HsPDA which failed to close on the first attempt. Surgical ligation is indicated if there was a failure to close after ≥2 courses of medication, or in those symptomatic cases with persisting complications from the shunt but with contra-indications to medications. We defined successful pharmacologic closure of PDA if there was the amelioration of clinical symptoms and signs of PDA and/or echocardiographic evidence of PDA closure within 72 hours of treatment.

Data Collection

After ethical approval, eligible neonates were identified from the NICU administrative electronic database and both electronic medical records and paper charts were reviewed. We abstracted demographic characteristics such as GA; BW; sex; antenatal steroids; mode of delivery; singleton vs multiple pregnancy; Apgar scores at birth; antepartum complications; score for neonatal acute physiology (SNAP-II); details of PDA; types of medications, dose, and duration of pharmacotherapy; side effects of treatment (i.e., acute kidney injury defined as urine output below 0.5 mL/kg/hour for above 8 hours or increase in serum creatinine above 30 µmol/L within 72 hours of treatment or above or equal to 50% from baseline, gastrointestinal bleeding), NEC above or equal to stage II by modified Bell staging,[14] surgical PDA ligation, IVH (based on validated classification system),[15] and BPD defined as the need for oxygen or respiratory support at 36 weeks postmenstrual age.

Standard BW *Z*-scores were calculated using Olsen nomograms and classified into three categories: Above −0.5, from −0.5 to −2.0, below−2 (severe IUGR).[16] Infants with *Z*-score above −0.5 constituted the reference group.

Outcome Measures

The primary outcome was the PDA closure rate following the first course of any of the three medications. Secondary outcomes included the proportion of infants needing a second or third course of medication, surgical PDA ligation, and those who had side effects from their pharmacologic treatment (such as gastrointestinal bleeding, intestinal perforation, acute kidney injury, NEC above or equal to stage II), BPD and death during the hospital stay.

Sample Size

A study by Madeleneau et al.[11] showed an 18% difference in the proportion of neonates who closed their PDA after the first course of NSAIDs between categories of infants with BW above −0.5 *Z*-scores, compared to those with BW below −0.5 *Z*-scores. A sample size calculation of 120 neonates in each group (BW category above −0.5 *Z*-scores and the IUGR categories) would be required to find a 20% difference in PDA closure rates between growth-restricted infants and AGA infants at 80% power and alpha error of 5%.

Statistical Analysis

The study cohort was summarized using descriptive statistical methods. We compared the baseline characteristics between the three groups using univariate analysis. Chi-squared tests or Fisher's exact tests for categorical variables were used where appropriate, and the student's *t*-test was employed for continuous variables. Statistical analyses were performed using stata 14 (Stata Corp, College Station, TX). Multivariable logistic regression analysis was performed to evaluate the independent association of BW *Z*-score and response to NSAIDs and subsequent PDA ligation. Covariates included in the regression model include factors that are associated with PDA closure (GA, antenatal steroids, gender), mode of delivery, and illness severity score. A two-tailed $p < 0.05$ was considered statistically significant.

Results

A total of 1,511 preterm neonates were admitted to NICU during the study period. Of which 769 (51%) neonates were diagnosed with PDA. A total of 517 neonates received at least one course of medical treatment for PDA (Flowchart 1). Of 517 included neonates,

Flowchart 1: Flow diagram of the study population

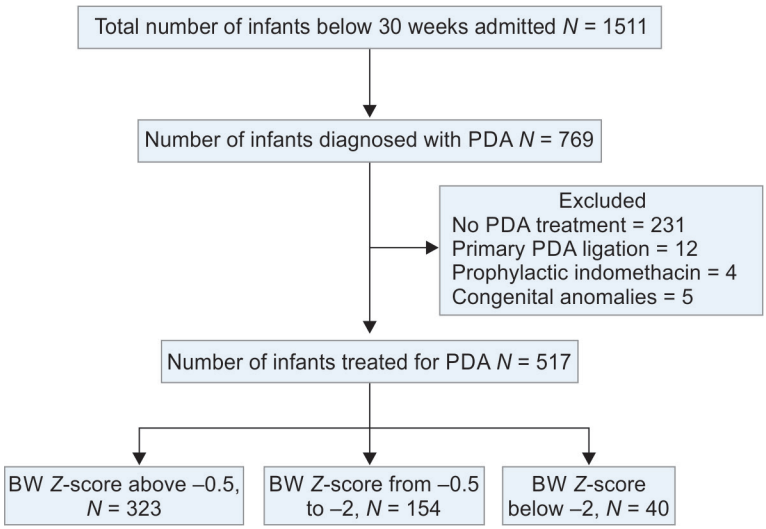

Table 1: Demographic characteristics

	BW Z-score above –0.5, N = 323	*BW Z-score from –0.5 to –2.0, N = 154*	*BW Z-score below –2.0, N = 40*	*p-value*
Maternal				
Maternal age (year)[a] mean	31.1 ± 5.8	31.9 ± 5.04	30.5 ± 5.5	0.145
Hypertension, *n* (%)	16 (4.9)	51 (33.1)	20 (50)	<0.001
Antenatal steroids, *n* (%)	278 (86)	140 (91)	40 (100)	0.01
Cesarean delivery, n (%)	193 (59.7)	103 (66.8)	35 (87.5)	0.002
Neonatal				
GA (week)[a]	25.9 ± 1.7	25.9 ± 1.85	26.6 ± 1.56	0.03
Birth weight (g)[a]	928 ± 222	713 ± 165	538 ± 90	<0.001
Male, *n* (%)	160 (49.5)	79 (51.3)	28 (70)	0.05
Multiples, *n* (%)	90 (27.8)	51 (33.1)	6 (15)	0.07
Apgar score at 5 minutes[b]	7 (6, 8)	7 (6, 8)	7 (5, 8)	0.30
SNAP-II[b]	14 (5, 24)	14 (9, 22)	14 (9, 22)	0.37

[a]Mean ± SD, [b]Median (interquartile range), SNAP-II, Score for acute neonatal physiology, version II

323 (62.5%) neonates had BW *Z*-score above – 0.5; 154 (29.8%) had from – 0.5 to –2.0, and 40 (7.7%) had below –2.

Table 1 shows the comparison of demographic characteristics. Maternal hypertension, exposure to antenatal steroids, and cesarean delivery were significantly higher in BW *Z*-score below –2 group. As expected, the mean GA and BW was significantly different among the three groups. Male gender was also higher among BW *Z*-score below–2 group.

Response to First Course of Medications

Of the 517 neonates, 206 (39.9%) received indomethacin, 301 (58.2%) received ibuprofen, and only 10 (1.9%) infants received acetaminophen as the first course of medication.

The PDA closure rate following the first course of NSAIDs was 51, 49, and 50%, respectively, among the three groups, which was not statistically significant (Table 2).

Patent Ductus Arteriosus Ligation

A total of 90 (17.4%) infants had surgical PDA ligation following medical treatment. There was a trend toward an increased rate of surgical PDA ligation rate with worsening growth restriction (15% vs 20% vs 25%). There was no significant difference in the complication rates and neonatal morbidity among the three groups except for BPD (Table 2). Bronchopulmonary dysplasia was also significantly higher among BW *Z*-score below –2 group as compared to normal growth neonates (85% vs 68%, $p = 0.04$).

Table 3 shows the multivariable logistic regression analysis adjusting for GA, sex, antenatal steroids, mode of delivery, and SNAP-II. There was no significant difference in PDA closure rate following the first course of NSAIDs between neonates with BW *Z*-scores below –2 and those with BW *Z*-score above –0.5, (aOR: 0.68; 95% CI: 0.33–1.39), and those with *Z*-score from –0.5 to –2.0 (aOR: 0.89; 95% CI: 0.59–1.35). However, the adjusted odds of PDA ligation were significantly higher among neonates with BW *Z*-score below –2 (aOR: 2.67, 95% CI: 1.12–6.34) but not among neonates with BW *Z*-score from –0.5 to –2.0 (aOR: 1.41; 95% CI: 0.84–2.39) as compared to BW *Z*-score above –0.5.

Discussion

Our study found a similar efficacy of the first course of NSAID medication in achieving closure of a PDA among the preterm growth-restricted neonates compared to well-grown neonates,

Table 2: Comparison of outcomes

	BW Z-score above –0.5, N = 323	*BW Z -score from –0.5 to –2.0, N = 154*	*BW Z -score below – 2.0, N = 40*	*p-value*
Primary outcome				
Response to the first course of NSAIDs, *n* (%)	164 (51)	75 (49)	20 (50)	0.91
Indomethacin, *n/N* (%)	69/126 (55)	39/64 (61)	10/16 (62.5)	0.65
Ibuprofen, *n/N* (%)	94/193 (49)	36/86 (42)	9/22 (41)	0.50
Acetaminophen, *n/N* (%)	1/4 (25)	0/4 (0)	1/2 (50)	0.33
Secondary outcomes				
Second course NSAIDs, *n* (%)	104 (32)	56 (36)	12 (30)	0.59
Third course NSAIDs, *n* (%)	27 (8.3)	15 (9.7)	4 (10)	0.85
Surgical PDA ligation, *n* (%)	49 (15)	31 (20)	10 (25)	0.17
Acute kidney injury, *n* (%)	31 (9.6)	19 (12.3)	3 (7.5)	0.74
Gastrointestinal bleed, *n* (%)	20 (6.1)	6 (3.9)	2 (5)	0.58
Spontaneous intestinal perforation, *n* (%)	8 (2.4)	7 (4.5)	3 (7.5)	0.18
IVH ≥ grade III, *n* (%)	41 (12.6)	19 (12.3)	1 (2.5)	0.16
NEC ≥ stage II, *n* (%)	33 (10.2)	19 (12.3)	7 (17.5)	0.35
BPD, *n* (%)	220 (68)	114 (74)	34 (85)	0.06

BPD, bronchopulmonary dysplasia; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; NSAID, non-steroidal anti-inflammatory drug; PDA, patent ductus arteriosus

Table 3: Adjusted outcome

Outcome	*Reference*	*BW Z-score from –0.5 to –2.0 (aOR; 95% CI)*	*BW Z-score below –2.0 (aOR; 95% CI)*
Primary outcome			
PDA closure after first course of NSAIDs	BW *Z*-score above –0.5	0.89 (0.59, 1.35)	0.68 (0.33, 1.39)
Secondary outcome			
PDA ligation	BW *Z*-score >–0.5	1.41 (0.84, 2.39)	2.67 (1.12, 6.34)

with closure rates between 49 and 51% for all three groups. However, the preterm growth-restricted neonates with *Z*-score below –2 with PDA had a 2.6-fold increase in odds of surgical PDA ligation as compared to normally grown neonates (*Z*-score above –0.5).

A common problem of studies assessing the potential association of growth restriction with adverse neonatal outcomes is that they do not differentiate between SGA and IUGR, even though the two terms are not synonymous.[17,18] A recent systematic review reporting the association of IUGR/SGA and PDA included 47 studies reported that there was no conclusive evidence of an association between growth restriction and PDA.[19] This finding may be due to marked heterogeneity across the studies in regard to the definition of growth restriction and PDA. For example, when the authors use the definition of SGA as BW less than 10 percentile, there was a significantly reduced risk of PDA (OR: 0.81; 95% 0.66–0.98) but this association disappeared when SGA was defined as BW less than 3 percentile (OR: 1.09; 95% CI: 0.7–1.71).[19]

In contrast to our findings of similar efficacy of PDA closure following the first course of any of these NSAIDs regardless of the birthweight *Z*-score, Madeleneau et al. reported that the success rate of the first course of ibuprofen decreases with increasing severity of growth restriction in France.[11] In their study of 185 neonates (GA from 24 weeks to 27 6/7 weeks), only 18 neonates were BW *Z*-score below –2. The success rate of the first course of ibuprofen was 45% in the study cohort. The risk of failure of the first course of ibuprofen increased with the degree of growth restriction, reaching above 12-fold higher risk of failure with a wide confidence interval (adjusted OR: 12.8; 95% CI: 2.3–70.5). However, there was no significant difference in surgical PDA ligation rate with increasing growth restriction. The two important differences between our study and the study by Madeleneau et al. is that our study had a higher number of SGA infants (194 infants below 30 weeks) as compared to 55 infants (GA below 28 weeks) in their study. Second, our cohort consisted of infants who receive indomethacin and ibuprofen as compared to ibuprofen treatment in their study.

Traditionally, studies on PDA management have often excluded SGA/IUGR subgroups. The SGA status is analyzed as a risk factor for PDA, rather than a predictive factor for treatment failure. In our study, we classified BW standard deviations (SDs) into three categories to reflect the potential continuous pathological effect of worsening growth restriction affecting mortality and neonatal morbidities. Previous epidemiologic studies have described preterm infants' growth restriction status using a –2 SD (or tenth percentile) cut-off for SGA. However, more recent studies show that this definition may no longer be appropriate because it inadequately describes the risks associated with fetal growth restriction based on just a single cut-off value.[20,21] Based on Delphi procedure, the growth restriction

in the newborn is defined as BW less than third percentile or the presence of three out of the 5 following; BW less than 10 percentile; head circumference less than 10 percentile; length less than 10 percentile; prenatal diagnosis of fetal growth restriction and maternal pregnancy information such as preeclampsia or pregnancy-induced hypertension.[18]

In preterm neonates, a persistent PDA is likely due to increased sensitivity of the immature ductus to prostaglandin E2 (PGE2). Also, PGE2 increases the intracellular concentration of cyclic adenosine monophosphate (cAMP), which activates cAMP-dependent protein kinase A (PKA), resulting in vasodilation in the ductus arteriosus. Prostaglandin E2 acts through three G protein–coupled receptors (EP2, EP3, and EP4) that activate adenylyl cyclase. The immature ductus has increased cAMP production because of increased binding of PGE2 to the individual EP receptors and increased potency of cAMP on PKA-regulated paththways.[22]

A previous study showed higher rates of significant PDA within 48 hours of birth among growth-restricted preterm neonates of 26–32 weeks in comparison to non-growth-restricted neonates (65% vs 40%).[8] Growth-restricted preterm neonates are at increased risk of elevated concentrations of inflammation-associated proteins by postnatal day 14.[23] Part of this increased risk might be a consequence of their greater tendency to be exposed to inflammatory stimuli such as sepsis/bacteremia, and prolonged ventilation. This inflammation and likely injury thereof might play a role in modulating response to NSAID treatment. Also, higher pulmonary blood flow is observed in growth-restricted preterm neonates resulting from lower pulmonary vascular resistance due to chronic hypoxia *in utero*. Catecholamines, especially norepinephrine has been shown to increase pulmonary blood flow in fetal lambs.[24,25] This pulmonary vasodilator effect of norepinephrine can be enhanced by glucocorticoids[26] production which is increased in fetuses with IUGR of placental origin.[27]

In a comparative study by Ibara et al., the histologic changes of PDA, such as fragmentation, coagulation necrosis of the intimal elastic lamina, hemorrhage with necrosis, and loosening of elastic fibers and muscles in the tunica media, were seen more frequently in IUGR neonates compared to AGA neonates.[28] These ductal changes may explain why HsPDA has been reported to occur more frequently and at an earlier postnatal age in very preterm growth-restricted neonates.[8,9] Reactivity to ductus arteriosus is also impaired by exposure to chronic fetal hypoxia.

We observed a 2.6-fold increase in surgical PDA closure among neonates with BW *Z*-score below −2. Similarly, Madelenau et al. from France reported a linear relationship between the degree of growth restriction and the need for surgical closure following ibuprofen treatment.[11] Compared to infants with *Z*-scores above −0.5, infants with *Z*-scores between −1.5 and −0.5 had more than 2-fold (OR: 2.6; 95% CI: 1.03–6.8) and infants with *Z*-scores below −1.5 had more than 12-fold (OR: 12.8; 95% CI: 2.3–70.5) increased odds of undergoing surgical closure following the first course of ibuprofen.[11] Boghossian et al.[12] reported higher rates of surgical PDA ligation among preterm SGA neonates after pharmacological treatment with indomethacin or ibuprofen. Compared to neonates with BW *Z*-score below −0.5, surgical PDA ligation following pharmacological treatment was higher among neonates with BW *Z*-score from −2 to −0.5 (OR: 1.23; 95% CI: 1.02–1.47) but this association was not significant for neonates with BW *Z*-score below −2 (OR: 0.83, 95% CI: 0.52–1.33).[12]

Ibuprofen is available as a racemic mixture of R- and S-ibuprofen, with S-ibuprofen being the pharmacologically active drug. The higher requirement of PDA ligation in more growth-restricted neonates might partly be explained by the fact that SGA neonates have been known to have a much higher clearance of S-ibuprofen compared to the appropriate weight for age neonates.[29] Currently used ibuprofen dosing regimens, however, do not consider the enantiomer-specific pharmacokinetics and postnatal day. The dosage of Ibuprofen needs to be adjusted based on BW and postnatal age.[29]

We also observed a significantly higher rate of BPD among growth-restricted infants. This association remained even after adjusting for PDA ligation (aOR 3.27; 95% CI 1.40–9.83). Our finding is similar to previous studies who reported SGA was associated with BPD.[30,31]

The strength of this study was the robust data over nine years from a tertiary care provincial referral NICU in Canada dealing specifically with the population of interest. We followed our PDA treatment guidelines and had a consensus between physicians about when to treat the ductus and with what medications. The pragmatic design of this study was very practical and can relate to the day-to-day practice where we target the HsPDA that is becoming problematic for our infants on an individual case-to-case basis. This goes in favor of the current individualistic approach that many physicians employ toward the ideal management of the ductus arteriosus.

Our study had the limitations of a retrospective design. This is a single-center study and there is an imbalance in the sample size for the three groups. As the study spans a period of nine years, there have been some differences in the relative use of one medication over other medications depending on drug availability locally and elsewhere in the world. There were some changes in ventilation management, especially using non-invasive ventilation during the latter part of the study period. Nonetheless, the treatment approach to PDA was relatively uniform throughout the study period.

Knowing that the commonly used NSAIDs have adverse effects, our study may help inform which neonates are less likely to respond to pharmacologic treatment. This study also highlights that in the smallest birthweight preterm infants, ductal closure is harder to achieve pharmacologically and may cause persistent adverse hemodynamic effects necessitating surgical ductal ligation more often. This might invoke further research into a deeper understanding of the pathophysiology of the underlying mechanisms and could also open new avenues of tackling the symptomatic ductus arteriosus in these infants differently. More research is also needed to elucidate the specific pathways and interactions of prostaglandins in mediating the ductal closure in growth restricted neonates and to identify other factors or unknown confounders that may play a crucial role as there is a paucity of current evidence on this crucial topic.

In conclusion, we observed a similar rate of PDA closure following the first course of NSAIDs between appropriately grown neonates and growth-restricted neonates. However, growth-restricted preterm neonates have higher odds of PDA ligation following pharmacological treatment as compared to appropriately grown preterm neonates with PDA.

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REVIEW ARTICLE

Quantum Cryptography for Securing Personal Health Information in Hospitals

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Received on: 03 November 2022; *Accepted on:* 10 November 2022; *Published on:* 23 December 2022

Abstract

Healthcare systems widely use information technology (IT) for system authentication (digital signatures), web surfing, e-mails, instant messaging, protecting data at rest, Voice over Internet Protocol (VoIP) telephony, and cellular telephony. To protect patient identification and healthcare information, cryptographic systems are widely used to secure these data from malicious third parties (adversaries). In our healthcare systems, we have had reasonable success in the efficient storage of the information of our patients and their families, in its timely retrieval, and in ensuring its safety from adversaries. However, the data are increasing rapidly and our current computational systems could be inadequate in the not-so-distant future. In this context, there is a need for novel solutions. One possibility can be seen in quantum computing (QC) algorithms/devices that can provide elegant solutions based on subatomic interactions. In this review, we have summarized current information on the need, current options, and future possibilities for the use of QC algorithms/devices in large data systems such as healthcare. This article combines peer-reviewed evidence from our own clinical studies with the results of an extensive literature search in the databases PubMed, EMBASE, and Scopus.

Keywords: Cryptographic systems, Health information, Healthcare, Hospital, Newborn.

Newborn (2022): 10.5005/jp-journals-11002-0043

Highlights

- In our healthcare systems, we have had reasonable success in the efficient storage of the information of our patients and their families, in its timely retrieval, and in ensuring its safety from adversaries. However, the data are increasing rapidly and our current computational systems could be inadequate in the not-so-distant future.
- In this article, we have reviewed possible solutions based on QC algorithms/devices that can provide elegant solutions based on subatomic interactions.
- Quantum cryptography focuses on protecting patient health information (PHI). During the transfer, data are first encrypted (encoded) and the recipient then decrypts (decodes) the information.
- Details of various methods of encrypting and decrypting have been provided. Current information on various protocols for QC has been summarized, and future possibilities have been discussed.

Introduction

Healthcare systems widely use IT for system authentication (digital signatures), web surfing, e-mails, instant messaging, protecting data at rest, VoIP telephony, and cellular telephony.[1–3] To protect patient identification and healthcare information, cryptographic systems are widely used to secure these data from malicious third parties (adversaries).[4,5] Several strong encryption algorithms are well-known, such as the secure hash algorithm (SHA)-1, SHA-2, triple data encryption algorithm system (TripleDES), advanced encryption standard (AES), message digest (MD)-5, and Rivest–Shamir–Adleman (RSA, named after the last names of Ron Rivest, Adi Shamir, and Leonard Adleman).[6–9] Conventional cryptographic algorithms have been used in our healthcare system, but these systems are now beginning to show limitations with the ever-increasing amounts of private information being accrued and produced.[7] These difficulties are particularly important in mother–infant and neonatal intensive care units (NICUs) as there is a need to secure the personal health information (PHI) that has been obtained from the whole family.[10,11]

In our healthcare systems, we have had reasonable success in the efficient storage of the information of our patients and their families, in its timely retrieval, and in ensuring its safety from adversaries.[12] However, the data are increasing rapidly and our current computational systems could well become inadequate in the not-so-distant future.[13] In this context, there is a need for novel solutions. One possibility can be seen in QC algorithms/devices that can provide elegant solutions based on subatomic interactions.[14] These devices resemble classical computers in the need for a defined input, and processing of data, and show a recognizable output, but do not need conventional digital semiconductor processors with interface busses and external networks.[14] Unlike conventional devices, a fully-functional QC algorithm/device might paradoxically show an exponential increase in its capacity to process

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How to cite this article: Mantry H, Maheshwari A. Quantum Cryptography for Securing Personal Health Information in Hospitals. Newborn 2022;1(4):333–339.

Source of support: Nil

Conflict of interest: None

data.[15–19] These should be able to handle the increasing workload in progressively smaller intervals of time that might eventually become nearly immeasurable.[13,14,19–22] Many of these devices currently do show high margins of errors, but encouragingly, many potential solutions can now also be seen.[23]

The QC models have brought exciting possibilities for outcomes prediction in many situations with large datasets, such as in hurricanes, global warming, forest fires, and pandemics.[24] These non-canonical prediction models have shown new possibilities for improving the efficiency and prediction of outcomes in our healthcare systems.[14,24] The QC systems can help analyze large, private patient datasets without the risks of decryption.[7,25] A staggering number of implausible events could possibly be solved if we can develop mechanisms to manage entropy related to multiple concurrent events and lower the error rates to levels that we tolerate in our current electronic semiconductor systems.[13,26–28] The only dilemma is whether we are ready in our technological quest for solutions to accept probabilities instead of certainties.[29,30] In this review, we have described the need, current options, and future possibilities for the use of QC algorithms/devices in large data systems such as healthcare.

Need

In the last two decades, technological advances in electronic medical records (EMRs), continuous monitoring of vital signs, telehealth, and affordable at-home testing devices have improved neonatal care.[31–33] With families' consent, sharing of the data obtained from these devices can improve efficiency in patient care and minimize errors.[32,34] Healthcare providers can utilize these real-time data not only to improve patient care but also for clinical research focused on recording outcomes and drug trials.[35] Families' satisfaction can also be recorded, and education can be more focused and improved. Diagnostics can also be evaluated with greater conviction by an improved recording of data and coordination between various medical subspecialties. Findings can also be analyzed better using newer modalities such as machine learning (ML). The entire health sector can become more data-driven.[35]

The concerns are that all the above-mentioned datasets contain the PHI of the patients in electronic health records (EHRs)/EMRs, medical devices, computers, the cloud, emails, servers, databases, and other associated systems.[5,36] These detailed data make the healthcare sector easy prey to cyberattacks.[5,17,36] The hospital systems and medical companies need to retain the trust of the infants' families by focusing on patient security and access to their data. The Health Insurance Portability and Accountability Act (HIPAA) is one important example of legislation that outlines the maintenance of PHI and the protection of identity from fraud/theft.[37–39] The HIPAA journal[40] reports an unsettling trend, showing a conspicuous rise in the number of healthcare records getting exposed every year.[41] According to the data breach statistics published so far, 2015 has been one of the worst years with more than 113.27 million records being exposed. Nobody wants to remember the infamous "WannaCry" malware attacks of May 2017 with data breaches in the British National Health Service and many reputable medical companies in the USA information.[42] Investigations showed loss of information such as dates of birth, credit card information, social security numbers, addresses, email IDs, and phone numbers, which were sold on the dark web; some patient records fetched up to US$1000. According to the US Department of Health and Human Services, such deliberate hacking accounts for about 75% of healthcare breaches.[43] The affected people continue to face the brunt for the rest of their lives.

Mother–infant units and NICUs are high-priority areas in hospitals where the PHI needs to be secured.[44] Infants and their families are a heterogeneous population, with varying capacities to protect their identifiers and their social, financial, and health information.[45] Mothers and other family members are at risk of developing transient psychological conditions which might affect their employability even after they have fully recovered.[46] Infants are a uniquely vulnerable population because of limitations in their legal rights and capacities for autonomous decision-making.[47] This means that special provisions are needed to ensure their protection from these risks, which include, but do need to extend beyond parental proxy consent on their behalf.[47,48] We also need special considerations in the storage of biomedical information because of the sensitive nature of such data, and the potential immediate and longer-term implications of PHI in the context of family dynamics.[48] These require immediate determinations about who has access to, and control over, the infants' PHI that can alter the life course of these children.[48,49]

Cryptography

Overview of Modern Cryptography

The term cryptography was derived from two Greek roots, *kryptos* meaning secret, and *graphein* meaning to study/write. The composite word, cryptography, refers to the art of securing private communications in presence of an eavesdropper or adversary.[50] Messages are secured by first "encrypting" the plain text into a cipher (a way of disguising in code) in a message that is then sent to the recipient.[51] The recipient "decrypts" the message from cipher to plain text using a tool for back translation, usually referred to as a "key."[52] This process reduces the risk of loss of important information. Cryptography is broadly classified into two categories: Private/symmetric key cryptography and public/asymmetric key cryptography.[53]

- **Private/symmetric key cryptography:** In private systems, a single key is used for both encryption and decryption, hence the name symmetric. In one experiment, one of two members of the team wants to send a sequence of bits, 0110100 to another with the shared key 1110101.[54] She/he encrypts the message using a bitwise "XOR" operation (a logical operation that stands for "exclusive or"). The encrypted message looks like 1000001. An eavesdropper who does not have access to the key fails to comprehend the message while the original recipient can decrypt it by applying the "reverse XOR" operation, yielding the message sequence bits 0110100. This is a classic example of a one-time pad encryption technique.
- **Public key cryptography:** Public systems are more complex than private key cryptography.[55] The team members use more than one key for sending different messages to reduce the chances of hacking. The public key may include two mathematically-related keys, one (public) used for encrypting that can be made freely available, and another (private) key that is protected and is needed for decrypting. The private key is usually derived using complex, more sophisticated mathematical systems. Besides the Diffie–Hellman key exchange protocol,[56] two other public key encryption techniques are the RSA and the Elliptic

Curve Cryptography (ECC).[57,58] Trapdoor functions that are easy to compute in one direction but not in the other, are used extensively to build public key cryptosystems.[59,60]

Advanced Encryption Standard

Advanced encryption standard is a kind of symmetric block cipher that cuts input data into chunks of fixed length and encrypts using a key.[61,62] This is currently being used in government agencies to protect the data encryption standard (DES), another symmetric key encryption algorithm that uses a key of only 56 bits.[63] Even though it is vulnerable to quantum attacks, higher AES key lengths with compounded complexity increase its safety.

Rivest–Shamir–Adleman Encryption

In the RSA encryption systems, it might be possible to create a public key such as the product of two large prime numbers, p and q.[64] The encoding value may be large, c. Since the prime numbers are kept secret, most observers will be able to encrypt a message but only an operator who knows the primes will be able to decrypt it. The security of RSA relies on the practical difficulty of factoring the product of two large prime numbers, which serves as its trapdoor function.[65]

Elliptic Curve Cryptography

Elliptic curve cryptography is the study of mathematical properties of elliptic curves, which are a set of points (x, y), where $y^2 = x^3 + ax + b$.[58] The variables a and b belong to a field K that may be made up of real, rational, or complex numbers. Fields are important algebraic structures that permit the application of certain operations on the members of the field. Elliptic curves use shorter keys to optimize memory storage.[66] For example, the security provided by a 256-bit key in ECC is comparable to a 3,072-bit key in RSA.

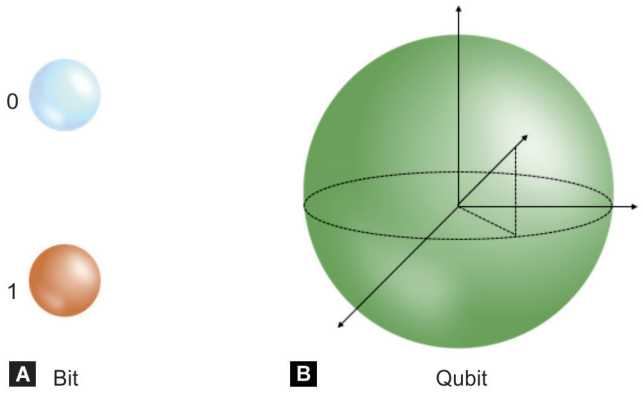
Quantum Computing

With the increasing number of transistors being used in a given chip, the speed of classical computers has increased but there are limits posed by the laws of quantum mechanics.[18,67] Classical computers are known to operate on a binary string of "bits," which are referred to as zeros and ones, and notated as "kets" (Dirac notations) $|0\rangle$ and $|1\rangle$.[68,69]

The key distinguishing feature of a quantum computer is referred to as a "qubit."[70] Figure 1 show a schematic representation of bits and a qubit. Each qubit is a superposition of two independent unit vectors in a 2-dimensional space and can be represented by the column vectors.[71] In other words, $|0\rangle$ and $|1\rangle$, which are independent unit vectors, would make our choice for the bases of the 2-dimensional vector space.[71] A $2n$ dimensional vector space would be having $2n$ basis vectors. In summary, a qubit state is a superposition of the two basis vectors such that the vector is normalized.[72]

Tensor Product

Tensor product (TP) results from an interaction between ≥2 qubit states. This concept helps us mathematically characterize the phenomenon of quantum entanglement (QE) (*vide infra*).[73] This needs to be differentiated from TensorFlow quantum (TFQ), which is a quantum ML library for rapid prototyping of hybrid quantum–classical ML models.[74]



Figs 1A and B: (A) A classical binary bit can only represent a single binary value, such as 0 or 1, meaning that it can only be seen in one of two possible states (off or on, false or true, low or high). Classical computing devices manipulate those bits with the help of logical gates (AND, OR, NOT); (B) In QC, a qubit or quantum bit is the basic unit of quantum information. It is a two-state quantum-mechanical system, represented by a superposition to achieve a linear combination of two states. Information is stored in quantum bits, or qbits. A qbit can be in states labelled |0> and |1>, but it can also be in a superposition of these states, a|0> + b|1>, where a and b are complex numbers. If we think of the state of a qbit as a vector, then superposition of states is just vector addition. Every extra added qbit can help store twice as many numbers. For example, with 3 qbits, it is possible to get coefficients for |000>, |001>, |010>, |011>, |100>, |101>, |110> and |111>

Quantum Entanglement

Quantum entanglement is a physical phenomenon seen in quantum physics, but not in classical mechanics. QE is seen when the physical properties of two particles such as position, momentum, spin, and polarization are perfectly correlated, even when these particles are separated by a large distance.[75] In this situation, the total spin of these two particles will be predictable.[76] Measurements of a particle's properties will result in an irreversible wave function collapse of that particle and will change the original quantum state, affecting the entangled system as a whole.[76]

Measurement Postulate

The MP in quantum mechanics pertains to the degree the wave function collapse occurs.[77] According to the Schrödinger equation, which describes the wave function in a quantum-mechanical system, the wave function evolves deterministically as a linear superposition on different states.[78] In other words, after one initial observation, all subsequent measurements remain consistent with these first-time observations.

No-cloning Theorem

This admits our inability to clone any arbitrary quantum state into multiple copies of itself.[79] If we could, this would have informed us about the behavior and properties of the state by applying different measurement operators to the state countless times. Despite all the measurements, we would always have information about the initial state.

Quantum Algorithms

Quantum algorithms are a set of instructions run on quantum computers similar to how classical algorithms are meant for classical computers.[80] The two most popular quantum algorithms are Shor's algorithm and Grover's algorithm.[81,82] Shor's algorithm is an algorithm for finding the prime factors of an integer using a specific unitary operator. Unfortunately, this algorithm can undermine the security of RSA and ECC due to program-related issues.

Modular arithmetic can provide insights into these algorithms. Grover's algorithm, also known as the quantum search algorithm, is a quantum algorithm that can reduce the time needed for an unordered search.[83,84] Simply put, an unordered search refers to searching for a particular element in a random list of elements such that no guess would bring us closer to the element we are looking for. The obvious way to do this would be to start from the first element and move onwards. Grover's algorithm can improve these searches as it is based on the properties of superposition, entanglement, and interference.[82] There is a special qubit gate called oracle which takes the input state and flips the phase of the chosen ket we are looking for and another gate which inverts the amplitudes of all the component kets about the mean of all the associated amplitudes.[85] However, all problems are still not resolved, and some limitations might appear when full-fledged quantum computers become a reality. Many algorithms such as Deutsch–Jozsa, Bernstein–Vazirani, Simon, quantum Fourier transform, quantum phase estimation, quantum counting, quantum walk search, and dense coding are being investigated.[86–91]

Quantum Key Distribution

The quantum key distribution (QKD) is a secure channel for encryption and decryption using the principles of quantum mechanics. The main tenets of quantum mechanics that makes QKD secure is the measurement postulate, where measurements of an unknown quantum system lead to a change in its state and any information about the initial state is lost after the measurement.[92] There are also possibilities of changes related to the no-cloning theorem and entanglement.

The BB84 Protocol

Named after its creators, Charles Bennett and Giles Brassard, BB84 is a quantum protocol used to generate a private key.[93] In this protocol, the first observer takes a series of qubits and performs any one of two orthogonal measurements on each qubit, such as the measurement of spin in the x and z directions. The first then send those to the second, who repeats the same job. The first operator, however, does not inform the second about which measurements were made and so the second operator will likely measure 50% of the qubits in the same manner as the first operator. After performing the experiments, they could publicly announce their readings and discard the measurements where they differ. The remaining set of measurements becomes their private key. An eavesdropper could then make major efforts to intercept the message qubit but due to the measurement postulate, she/he will be changing the qubit nearly 50% of the time. The no-cloning theorem suggests that she/he will not be able to copy these either. The original two operators will be able to publish a subset of their results and using the correlation they will be able to determine whether there has been any meddling with their key.

The E91 Protocol

This is a slight variation of the BB84 protocol and uses entanglement.[94] The first operator prepares several entangled qubits[95] and sends those to the second; she/he will keep one qubit and send the entangled partner to the second operator. The rest of the protocol resembles BB84. However, it is worthwhile to note that the first operator will not have to tabulate the measurements as the "correlatedness" of the entangled pairs will be certain.

Future Possibilities

Shor's algorithm suggests that many public key encryption techniques like ECC and RSA that are based on factoring and discrete logarithmic problems will remain considerably insecure in the face of QC.[96] However, there are a few quantum-safe encryption techniques today that would last at least for the next century, even if QC becomes a reality in the next 2–3 decades. The National Institute of Standards and Technology had recently listed four encryption methods that are ready for the post-quantum world: Cryptographic Suite for Algebraic Lattices (CRYSTALS)-Dilithium (a lattice-based signature scheme), a cryptographic signature algorithm FALCON, SPHINCS+ (a stateless hash-based signature scheme, which advances the SPHINCS signature), and CRYSTALS-Kyber.[97–99] Active research is going on developing lattice cryptography, multivariate cryptography, code-based cryptography, supersingular isogeny key exchange protocol, and symmetric key systems like AES and SNOW- 3G.[100–104] Campagna recently postulated that there will be three main questions about the number of years needed to fulfill our health sector needs: (a) Our encryption to be secure; (b) to make our IT infrastructure quantum-safe; and (c) before a large-scale quantum computer will be built.[105] The physical hardware required to build qubits includes transmons and superconductivity traps, and we will also need insights into cavity quantum electrodynamics.[13,106,107] Significant efforts are also being propagated toward developing topological quantum computers. On a positive note, researchers have recently built the world's largest functioning QKD network using photons and relay optics.[108]

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REVIEW ARTICLE

The Phylogeny, Ontogeny, and Organ-specific Differentiation of Macrophages in the Developing Intestine

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Received on: 03 November 2022; *Accepted on:* 10 November 2022; *Published on:* 23 December 2022

Abstract

Macrophages are large highly motile phagocytic leukocytes that appear early during embryonic development and have been conserved during evolution. The developmental roles of macrophages were first described nearly a century ago, at about the time these cells were being identified as central effectors in phagocytosis and elimination of microbes. Since then, we have made considerable progress in understanding the development of various subsets of macrophages and the diverse roles these cells play in both physiology and disease. This article reviews the phylogeny and the ontogeny of macrophages with a particular focus on the gastrointestinal tract, and the role of these mucosal macrophages in immune surveillance, innate immunity, homeostasis, tissue remodeling, angiogenesis, and repair of damaged tissues. We also discuss the importance of these macrophages in the inflammatory changes in neonatal necrotizing enterocolitis (NEC). This article presents a combination of our own peer-reviewed clinical and preclinical studies, with an extensive review of the literature using the databases PubMed, EMBASE, and Scopus.

Macrophages were first described at the beginning of the previous century by Paul Ehrlich and Ilya Metchnikoff as important mediators of innate immunity.[1] The name "macrophages" or "big eaters" came from the Greek words, "makros" or large, and "phagein" or eat.[2] Macrophages are large cells with an irregular cell shape, oval- or kidney-shaped nucleus, cytoplasmic vesicles, central nucleus, and high cytoplasm-to-nucleus ratio.[3] These cells are highly phagocytic and motile, and modulate immune responses by releasing various mediators.[4] The term mononuclear phagocyte includes lineage-committed bone marrow precursors, circulating monocytes, resident macrophages, and dendritic cells (DCs).[5] In this review, we have focused on the macrophage lineage as it expands and matures first, *in utero*, and plays an important role in the innate immune responses of newborn infants.

Keywords: Blood counts, Inflammation, Macrophages, Monocytes, Organ injury, Signaling.

Newborn (2022): 10.5005/jp-journals-11002-0044

Key Points

- Macrophages are large highly motile leukocytes with important roles in innate immunity.
- During evolution, many precursors to macrophages, such as amebocytes, coelomocytes, and hemocytes, have been identified.
- In the developing human embryo, macrophage precursors are identified in the yolk sac, the pools of erythro-myeloid progenitors, endothelial precursors, and later in hematopoietic stem cells.
- Macrophages play major roles in tissue homeostasis, innate immunity, inflammation, tissue repair, angiogenesis, and apoptosis of various cellular lineages.
- The differentiation of monocytes into inflammatory M1 and immunoregulatory M2 macrophages is a subject of active investigation.
- The gastrointestinal tract contains the largest reservoir of specialized macrophages. Small intestine and colon contain multiple, distinct macrophage subsets.
- Recognizing the heterogeneity of gut macrophages in premature and term infants raises an exciting possibility that targeted augmentation or depletion of subsets might be therapeutically useful.

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How to cite this article: Maheshwari A. The Phylogeny, Ontogeny, and Organ-specific Differentiation of Macrophages in the Developing Intestine. Newborn 2022;1(4):340–355.

Source of support: National Institutes of Health awards HL124078 and HL133022 to AM.

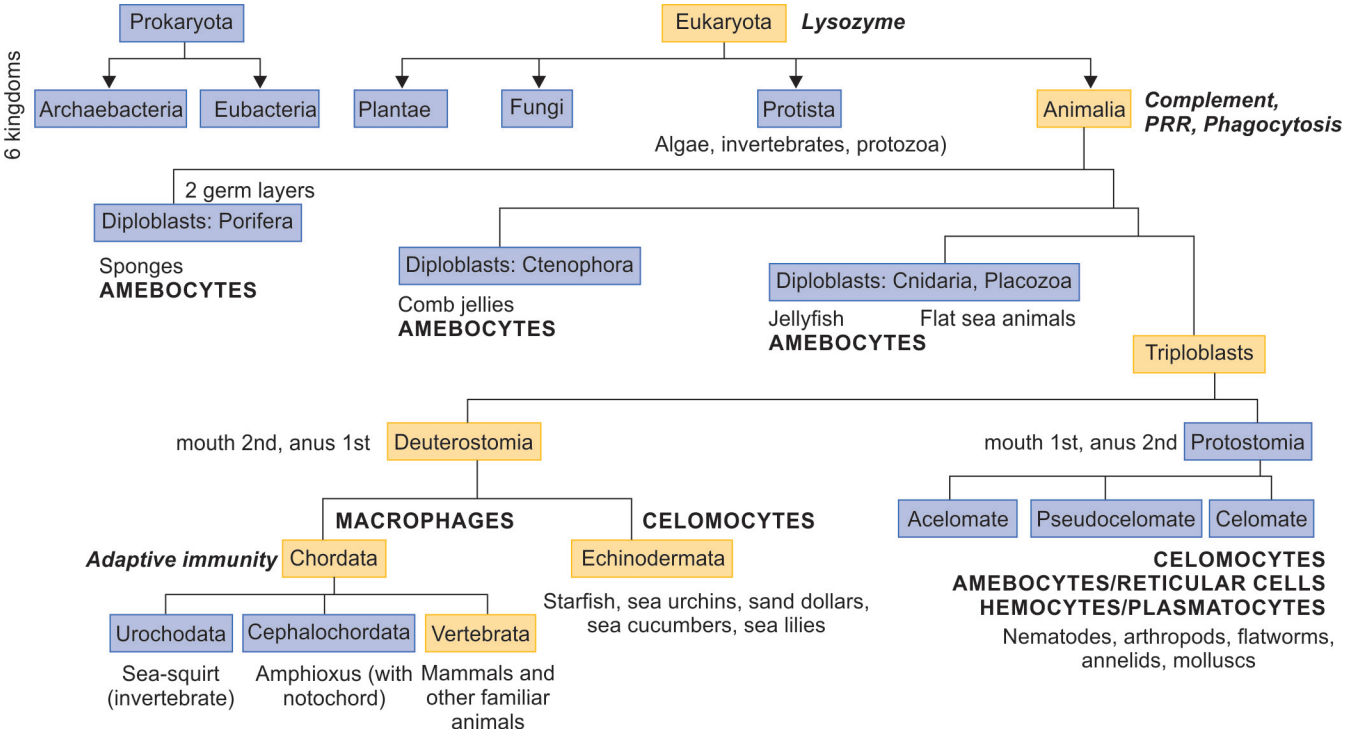
Conflict of interest: None

Evolution of Macrophages

Macrophages have Developed in a Conserved Process through Evolution

Macrophages can phagocytose smaller organisms, foreign materials, and cellular debris.[6,7] This ability to ingest particles larger than 0.4 µm in diameter has followed a recognizable pattern across evolution, be it in unicellular Protists such as the soil-living amoebae *Dictyostelium discoideum* and protozoans such as *Trichomonas vaginalis*, or in macrophages in multicellular eukaryotes.[8,9]

Flowchart 1: Phylogeny of macrophages. Schematic figure shows the development of macrophage-like cells and macrophages across evolution. The orange-colored boxes with eukaryota, animalia, triploblasts, deuterostomia, chordata, and vertebrata traces the evolution of vertebrate animals such as humans. The green font indicates key events in the development of immunity. Upper-case, red-font labels show the evolution of phagocytes. For each category of animals, one or more representative organisms are listed below in black font



Phagocytic cells in invertebrate species are known by other names such as amebocytes, celomocytes, or hemocytes, but each shows morphological similarities with the macrophages of vertebrates (Flowchart 1).[10,11] These similarities extend to the molecular level with the expression of proteins containing the scavenger receptor cysteine-rich (SRCR) domains.[6,12]

In diploblastic animals, the cellular endo- and ectoderm are separated by a gelatinous matrix (mesoglea) that contains motile amebocytes, which ingest and digest food caught by enterocytes and then transport the nutrients to the other cells.[8] These cells also promote innate immunity with pattern-recognition receptors (PRRs) and pore-forming proteins such as the macrophage-expressed gene 1 protein (Mpegl).[13]

In triploblasts, which represent the next stage in evolution, the middle layer is composed of mesodermal cells instead of the mesoglea but it contains similar phagocytes.[14] The two subclasses, the Protostomia and the Deuterostomia, are named based on sequence of the development of the gastrointestinal tract openings.[15] Vertebrates are a subclass of this group.[16]

Macrophage-like Cells in Triploblastic Prostomes with a Celomatic Cavity

In celomatic animals, an inner mesenteric layer holds the gut in the central cavity, and this promotes somatic growth.[10] The circulatory system further helps in increasing body size through efficient diffusion of gases and nutrients, and the removal of metabolic waste products.[17] This vascular system contains circulating macrophage-like defense cells known as the celomocytes/hemocytes. Similar cells, the plasmatocytes, have been identified in the corresponding ontogenic stage in developing fruit flies (*Drosophila melanogaster*).[18]

Blood vascular systems may be structured in one of two principal designs: open or closed.[17] In the open circulatory system of arthropods and noncephalopod molluscs, the circulating hemolymph empties from a contractile heart and major supply vessels into the hemocele body cavity where it directly bathes the organs. Annelids like the earthworm, cephalopods, and nonvertebrate chordates have a closed circulatory system, where the intestinal surface is in contact with soil microorganisms.[19] The are two freely circulating subpopulations of phagocytes, the autofluorescent eleocytes and amebocytes celomocytes.[20] Amebocytes, not eleocytes, express PRRs and the Toll-like receptor (TLR) signaling pathway.[21] These cells also express the oxidative stress-related superoxide dismutase and the antimicrobials lysozyme and lumbricin.[22]

Macrophage-like Cells in Deuterostomes such as the Chordates and its Constituent Vertebrates

Deuterostomes include the subclasses Chordata and Echinodermata (Flowchart 1).[23] One of the chordate subphyla is comprised of the vertebrates. In the vertebrate intestine, the mononuclear phagocyte system has three cellular lineages: monocytes, macrophages, and DCs,[24] that are released from the bone marrow.[25,26] The other phylum, Echinodermata, also contains circulating macrophage-like phagocytes.[27–31]

In zebrafish, embryonic macrophages migrate from the mesoderm into the spleen and gut.[32,33] The macrophages express tumor necrosis factor (TNF) to regulate the expression of mediators such as interferon regulatory factor 8 (IRF8), the complement Clq genes, and the G-protein-coupled receptor 35 and shape the gut microbiota. Macrophages also promote

intestinal lymphangiogenesis thorough the vascular endothelial growth factors.

Amphibian macrophages promote immune defenses, homeostasis, and tissue remodeling.[34] Chemokines such as the CXC ligand 12 (CXCL12) stimulate granulocyte/macrophage precursors to migrate from the liver to the bone marrow.[35] Macrophage differentiation is controlled through binding of the colony-stimulating factor-1 (CSF-l) to its cognate CSF-l receptor (CSF-1R) on committed macrophage precursors. IL-34 also binds CSF-IR and promotes differentiation into morphologically and functionally distinct macrophages.[36]

In reptiles, macrophages are seen in the lamina propria but can migrate into the subepithelial lymphoid aggregates.[10] Birds also have a well-developed mucosal immune system.[37] The gut lamina propria contains innate immune cells such as macrophages, although the differences with DCs have not been reported in detail. These cells express many PRRs. Early-life microbial colonization is critical for immunological maturation. Macrophages are involved in antigen uptake and protect against invading pathogens.[38,39]

Mammals show macrophage maturation in patterns that resemble those in humans (as described below). Obviously, there are important species-specific differences in the timing in gestation (day of pregnancy) and the relative importance of specific genes or genetic isoforms.

Development of Macrophages in Humans

Macrophage development has three different phases during the embryonic, fetal, and neonatal period (Flowchart 2).

Macrophage Differentiation in the Embryo

- *Lineage-restricted progenitors in the yolk sac (YS):* Hemocytoblasts with myeloid characteristics are first seen in blood sinuses in the secondary YS on day 18.[40] On day 19, some large-sized histiocytes, which is a term for tissue-resident macrophages, can be seen.[41–44] Two distinct macrophage lineages appear at 5 weeks; a larger MHC II-neg fraction appears first in the YS, mesenchyme and the fetal liver, and then in the thymic cortex, lymph nodes, splenic red pulp, and the bone marrow.[45] A minor population of MHC II^+ cells can be seen in the liver at 7–8 weeks, lymph nodes at 11–13 weeks, the thymic medulla at 16 weeks, and then in the gastrointestinal tract. These MHC II^+ cells then expand gradually.[45,46] Yolk sac-derived macrophages are independent of the transcriptional factor c-Myb during development, but depend on the transcriptional factor PU.1.[47]
- *Erythro-myeloid progenitors (EMPs):* On day 25, the YS and the embryo show EMPs developing from the capillary endothelium.[48] These cells proliferate and differentiate into macrophages by day 30 and then migrate to all embryonic organs except the central nervous system (CNS).[49]
- *Endothelial precursors in the aorta-gonad-mesonephros (AGM) zone*: The vascular endothelium in this zone produces $CD34^+$ $CD45^+$ hematopoietic stem cells (HSCs),[50] which differentiate into common myeloid progenitors (CMPs) and then into tissue macrophages either directly or via a monocyte stage. These macrophages migrate to all embryonic organs, except the CNS.[49]
- *Microglial precursors*: Some cluster of differentiation (CD) 45^+ $CD34^+$ myeloid cells located near the dorsal aorta migrate around day 30 into the CNS and differentiate into microglial precursors.[49,51,52]

Most macrophages in fetal organs develop from EMP and AGM progenitors.[51,52] Some EMPs in the liver differentiate directly into Kupffer cells without passing through a monocyte stage.[53] However, both these EMP-derived lineages are eventually replaced by BM-derived macrophages.

Macrophage Differentiation in the Liver

On day 32, some $CD45^+$ $CD34^+$ HSCs migrate from the AGM zone to the liver[51] and then differentiate into monocytes and macrophage precursors in the 8–20 weeks period. These cells comprise nearly 70% of all hematopoietic cells in the fetal liver, but then involute during the 20–23 weeks period when the erythroid cell pool begin to expand.[54] Some of these macrophages may arise from EMPs or from the $CD34^+$ $CD45^-$ hemogenic endothelial cells that produce $CD33^+$ macrophage precursors.

Monocyte development in the bone marrow followed by tissue migration and macrophage differentiation: While some $CD34^+$ $CD45^+$ HSCs migrate from the AGM zone into the liver, others migrate on the same day into the bone marrow. These cells remain detectable at 1 in 60 $CD34^+$ $CD45^-$ cells even at 24 weeks' gestation.[54] In the monocyte–macrophage lineage, the differentiation shows several discernible stages, including the CMPs, granulocyte–monocyte precursors (GMP), common monocyte and DC precursors (MDP), premonocytes (committed monocyte progenitors), and then monocytes.[55] These are all noticeable by the 7th week of gestation. After birth, the HSCs migrate from the liver to the bone marrow and mature as part of "definitive" hematopoiesis.[56]

There are three subsets of differentiating monocytes, a classification recognized by the nomenclature committee of the International Union of Immunologic Societies:

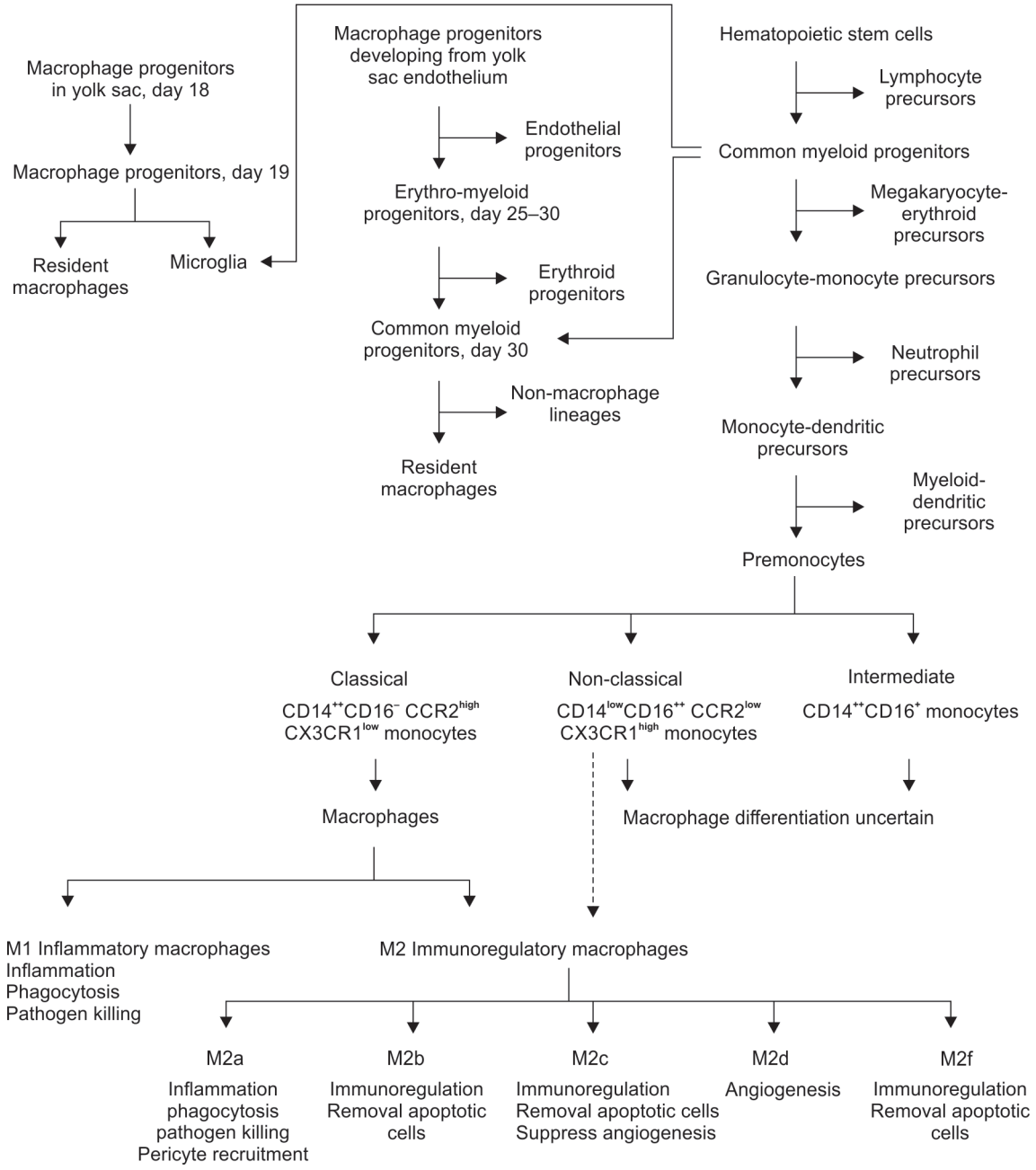
- *Classical, $CD14^{++}$ $CD16^-$ cells* (80–90%), which express CCR2, CD64, and CD62L. These show strong phagocytic activity, rapid responses to TLR ligation, and express inflammatory cytokines and reactive oxygen species (ROS) to recruit other leukocytes.[57]

In mice, classical monocytes ($Ly6C^{++}$) differentiate into macrophages in most organs[58] except in the gut, which has its own precursor cells.[3] However, these cells are uniform precursors of inflammatory macrophages in disease states in all organs. Some $Ly6C^{++}$ "tissue monocytes" in nonlymphoid organs may serve as effectors without differentiating into macrophages or DC, and present antigens to T-cells.[59] A subgroup of $Ly6C^{++}$ monocytes released from the marrow may also serve in a diurnal "anticipatory inflammation" regulated by the circadian gene Brain and Muscle ARNT-like 1 (BMAL1), as an innate response to frequently occurring environmental challenges.[60]

- *Non-classical, $CD14lowCD16^+$ cells* (10%) that lack CCR2, but express the Fc γ receptors CD64 and CD32.[61] These monocytes patrol the blood vessels and respond via a TLR7-triggered pathway to remove senescent endothelial cells.[62] Some cells extravasate to promote tissue healing, but show limited phagocytic activity and inflammatory responses to bacterial products.[61,63]

Murine studies show the corresponding $Ly6C^-$ cells to be terminally differentiated.[58] These cells are regulated by the transcription factor Nuclear Receptor Subfamily 4 Group A Member 1 (NR4A1).[64] Some of these $Ly6C^-$ monocytes might directly develop from MDPs in the marrow.[65] $Ly6C^-$ monocytes exhibit a longer half-life of 5–7 days, longer than the 8-hour lifespan of $Ly6C^+$ cells.[66,67] $Ly6C^-$ monocytes may be also be seen as terminally differentiated resident macrophages in blood or the "vasculature macrophages,"

Flowchart 2: Ontogeny of gut macrophages. Schematic figure shows the development of the three major categories of macrophages in the human embryo, from progenitors in the yolk sac (YS), from the YS endothelium, and from the hematopoietic stem cells (HSCs). A subset of the HSCs evolves into monocytes, and current understanding suggests the classical CD14^{++} monocytes differentiate into the M1 inflammatory and M2 immunoregulatory macrophages. There is a possibility that the CD14lowCD16 monocytes may (also) evolve into the immunoregulatory macrophages, which is shown as a broken arrow. Increasing information suggests that the M2 macrophages may be a heterogenous group comprised of multiple subsets



rather than *bona fide* monocytes.[68] Indeed, the primary function of these cells seems to be to patrol the vascular endothelium and monitor its integrity.

- *Intermediate cells that express both CD14 and CD16*.[68] A variable number of such cells express MHC-II, present antigens, and activate T lymphocytes. The exact role of these cells is not clear.

Monocytes are limited to blood, bone marrow, and spleen. These cells first appear in blood during the 5th month of gestation,[54] but comprise only a small proportion of the cellular lineages until the bone marrow becomes the predominant site of hematopoiesis.[69] Monocyte concentrations in the blood rise between 22 and 42 weeks; these cells comprise 3–7% of hematopoietic cells at 30 weeks[70] and there is a relative monocytosis at term with counts between 300 and 3300/μL (median 1400/μL). After birth, absolute monocyte counts rise during the first 2 weeks and then begin to drop in the 3rd week.[71–73] After leaving the bone marrow, monocytes circulate for 1–3 days and then move into tissues to differentiate into macrophages or into myeloid dendritic cells (mDCs). Both monocyte-derived macrophages (MDMs) and mDCs

are involved in a variety of immune functions such as phagocytosis, antigen presentation and cytokine production.[74] During the fetal/neonatal period, monocytes frequently develop epigenetic changes such as DNA methylation, microRNA expression, or histone modifications, that alter the immune pathways.[75] For instance, decreased trimethylation of lysine 4 on histone 3 (H3K4me3) alters the maturation of neonatal monocytes.[76]

In infants, monocytes are important determinants of innate immunity. There are several aspects to consider:

- *Maturity in movement*: By term gestation, monocytes attain considerable maturity in movement needed in trans-endothelial migration, chemotaxis, phagocytosis, and respiratory burst. Unlike neutrophils, cord blood monocytes show adherence, random migration, chemotaxis, phagocytosis, bactericidal activity.[77]
- *Maturity in pathogen elimination*: Neonatal monocytes generate superoxide anion (O-2) and hydrogen peroxide at levels comparable to those from adults.[78–80] Fetal and neonatal monocytes can kill pathogens such as *S. aureus*, *S. epidermidis*, *E. coli*, and *C. albicans* similar to those from adults.[78,81]
- *Maturity in inflammatory responses:* Neonatal monocytes produce cytokines such IL-1β, IFN-α and TNF at levels comparable to adults, but not IFN-γ, IL-8, IL-10, and granulocyte–macrophage colony-stimulating factor (GM-CSF). These cells also produce less extracellular proteins such as fibronectin, and bioreactive lipids like leukotriene B_4.[82–86]

As in amphibians and other phyla, monocyte differentiation into macrophages is controlled through binding of the colony-stimulating factor-1 (CSF-I) to its cognate CSF-I receptor (CSF1-R) on committed monocytes.[87] IL-34, which is expressed mainly in the skin and the CNS, also binds CSF-IR.[88] These ligands promote the differentiation of myeloid precursors into morphologically and functionally distinct macrophages.

Classification of MDMs

Monocyte-derived macrophages are comprised of two major phenotypes, the classically activated M1 and an alternatively activated, immunoregulatory M2 phenotypes (Flowchart 2).[89] The M1 and M2 subpopulations could represent distinct differentiation paths of a common precursor, or could represent a maturational pathway where the M1 phenotype transitions to M2 with loss of CD14 and increased expression of CD16 and other markers. Based on murine models with progressive loss of Ly6C, the latter possibility seems more likely.[90] The two subgroups show different surface markers, tissue localization, function, and intracellular signaling.

(a) *M1 macrophages* respond to cytokines such as TNF and interferon-γ, bacterial lipopolysaccharide (LPS), and the granulocyte–macrophage colony-stimulating factor (GM-CSF). These cells express CD54, CD80, CD86, and CD197.[91,92] In most situations, M1 macrophages are more efficient at phagocytosis and bactericidal functions;[93] *in vitro*, macrophages are activated toward an M1 functional program by infectious microorganism-related molecules and by inflammation-related cytokines TNF or IFN-γ.[90] M1 macrophages are characterized *in vitro* by an IL-12^{hi}IL-23^{hi}IL-10^{lo} phenotype; are efficient producers of toxic effector molecules (ROS and NO) and inflammatory cytokines (IL-1β, TNF, IL-6); participate as inducers and effectors in polarized Th1 responses.[90] These cells show high level expression of the CC chemokine receptor 2 (CCR2) and low levels of the CX3C-chemokine receptor 1 (CX3CR1).[90] Accordingly, the chemokine CCL2 recruits $CD14^+$ human/$Ly6C^+$ murine monocytes to inflammatory sites.[94]

Most M1 macrophages die, killed by their own NO production.[95] In an experimental acute lung injury model, these cells undergo Fas-mediated death, while the resident alveolar cells persist. M1 is likely a terminal differentiation phenotype, but some can undergo phenotype conversion to become tissue-resident macrophages. Macrophage polarization may be both transient and plastic.[96] The patrolling monocytes respond to the CX3C-chemokine ligand 1 (CX3CL1; human fractalkine/murine neurotactin), a chemokine present both in soluble and membrane-bound forms expressed on endothelial cells and in tissues.[97]

(b) *M2 macrophages* may respond more strongly to IL-4, IL-10, IL-13, and IL-21, and to glucocorticoids, and express high levels of surface scavenger receptors such as CD163, CD204, and the mannose receptor, CD206.[91,92] Accordingly, these cells show low-level expression of the CC chemokine receptor 2 (CCR2) and high levels of the CX3C-chemokine receptor 1 (CX3CR1).[98]

M2 macrophages are active in immunoregulation, maintain tissue integrity following injuries and in chronic infections, and promote angiogenesis.[93] The M2 macrophages are a relatively heterogeneous group, comprised of at least 5 sub-categories (M2a, M2b, M2c, M2d, and M2f).[4,99]

In vitro, M2 polarization has been noted in response to the concomitant activation of Fcγ receptors and TLRs, and to exposure to immune complexes and to anti-inflammatory molecules such as IL-10, TGF-β, and glucocorticoids.[89] M2 cells are characterized by an IL-12^{lo}IL-23^{lo}IL-10^{hi}TGF-β^{hi} phenotype and generally have high levels of scavenger, mannose, and galactose-type receptors.[58,100] In general, these macrophages take part in polarized Th2 responses, dampening of inflammation, tissue remodeling, angiogenesis, and immunoregulation.[101]

Tissue-resident macrophages are maintained locally by proliferative self-renewal, and retain an M2-like phenotype, for example, in the peritoneal cavity, brain, and lung.[102] The proliferation rates are low in steady-state conditions, but increase under inflammatory challenges.[103] Monocyte-derived macrophages may have fates similar to tissue-resident macrophages with maintenance of M2-like phenotypes and a low self-renewal capacity.[58] A number of cells probably die during inflammation, where the extent of survival possibly depends on the nature and magnitude of the insult.[58]

Generally, monocyte/macrophage development, differentiation, and proliferation are driven by macrophage colony-stimulating factor (CSF-1), GM-CSF, and cytokines such as IL-4 and IL-34.[104] CSF-1 is constitutively produced by mesenchymal cells, and it promotes the maturation of $Ly6C^+$ monocytes to $Ly6C^-$.[105] CSF-1 also increases macrophage proliferation with a negative feedback loop via macrophage production of CCL2.[58] It helps maintain the macrophage pool in the gut, kidney, peritoneal cavity, BM and hence in circulation, but not in the liver. It promotes M2 polarization. During inflammation, GM-CSF is the primary driver of hematopoiesis and it promotes the proliferation of M1-polarized MDMs.[106]

Dendritic cells (DCs) are a leukocyte subset specialized for antigen-presenting function. Similar to macrophages, DCs also originate early from a common granulocyte–monocyte–dendritic cell progenitor[107] in the YS, mesenchyme, and the liver at 4–6 weeks

of age.[108] DC precursors differentiate into:[109,110] (1) myeloid DCs (or mDCs), $CD11c^+$ cells that express myeloid markers such as CD13, CD33, CD1a-d, and CD11b; and (2) plasmacytoid DCs (or pDCs), $CD11c^-$ cells with a plasmacytoid morphology with well-developed rough endoplasmic reticulum and Golgi apparatus.[111] However, DCs mature most after birth[112] Neonatal DCs comprise about 0.3% of all mononuclear cells.[113] Compared to adults, neonatal pDCs exhibit low expression of ICAM-1 and MHC antigens and of co-stimulatory molecules CD40, CD80 or CD86, show a less efficient maturational response and immunostimulatory function,[114,115] Neonatal DCs express, and are less efficient in lymphocyte activation than those from adults.[114]

Determinants of Macrophage Polarization

In inflamed tissues, different subtypes of macrophages can be seen. The mechanisms are unclear, but such variability in polarization may arise from the presence of conserved lineages (rooted phenotypes), plasticity (possible to shape/mold), or flexibility (possible inter-convertibility). Here are these three possibilities:

(1) *Conserved lineages*: resident macrophages derived from YS or hepatic precursors are hypo-inflammatory and retain tissue-protective and reparative functions, whereas MDMs may evolve into different lineages.[5,116] For instance, human $CD14^{++}$ inflammatory monocytes and murine $Ly6C^+$ monocytes may differentiate into M1 macrophages.[117] Human $CD16^+$ or murine $Ly6C^-$ monocytes may differentiate into resident tissue cells (if not from transdifferentiation of M1 macrophages, *vide infra*).[58,118]

(2) *Plasticity related to phases of inflammation/specific tissues*: monocytes recruited soon after the onset of inflammation may differentiate into M1 macrophages, and those recruited during disease resolution may develop M2 characteristics.[119,120] In some tissues such as the intestine, extracellular matrix (ECM) contents such as transforming growth factor (TGF)-β and other mediators such as IL-10 can convert monocytes into hypo- or noninflammatory macrophages.[121]

(3) *Flexibility related to phases of inflammation/microenvironment*: macrophages may retain the ability to switch from one phenotype to the other depending on the microenvironmental stimuli.[122] M2 macrophages can be activated into M1 following exposure to TLR ligands or IFN-γ.[122] M1 macrophages might acquire M2 properties during resolving inflammation with increased sensitivity to ECM components such as TGF-β, although further evidence is needed that these changes do not get interrupted by nitric oxide.[123] Another example of such a phenotypic switch is with repeated exposures to LPS, which can induce *endotoxin tolerance* with a global switch in gene expression program.[123]

Monocytes/Macrophages Can Carry and Present Antigens in Lymphoid Tissue

Some monocytes that enter inflamed tissues do not differentiate into macrophages, but are able to phagocytose antigen(s) and carry those to naïve T-cells in lymph nodes. Tissue macrophages can also present antigens to the maturing adaptive immune system. M1 MDMs may present antigens and activate/polarize effector Th1 and Th17 cells upon production of IL-12 and IL-23, respectively. The TNF superfamily and the TNF receptor superfamily are likely involved, but not co-stimulators such as CD80, CD86, and CD28. Similarly, M2-like tissue macrophages, which produce TGF-β and express the αVβ8 integrin may be involved in the polarization of regulatory T cells.

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- Tissue-resident macrophages and MDMs can recognize microbial or damage-associated molecules for enhanced recruitment and differentiation of circulating monocytes into M1 macrophages.
- Tissue monocytes are recently described cells that can take up antigens in the tissue and move to lymph nodes, where they are able to present antigens to naive T-cells.
- After resolution of an acute inflammatory illness, memory macrophages or monocytes may be retained. These cells are functionally programed by a previously stimulus for either altered cytokine production to optimize the immune response depending on the type/concentration of the immune stimulus.

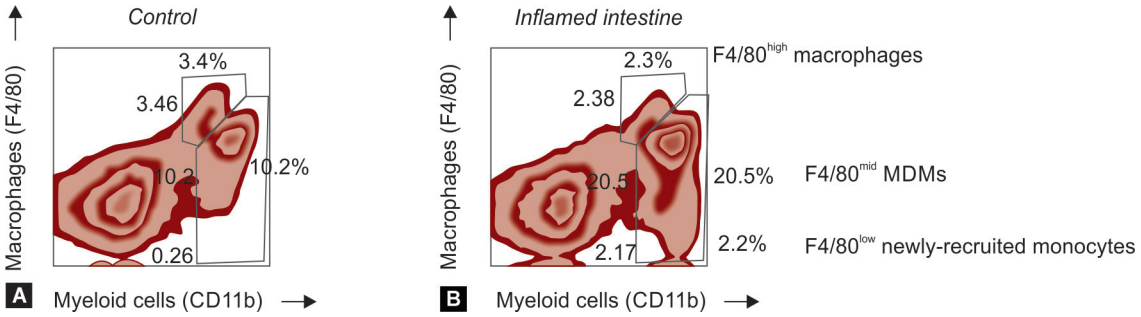
Gut Macrophages in the Developing Intestine are a Specialized, Hyper-inflammatory Cellular Population

The gastrointestinal tract contains the largest reservoir of macrophages in the body.[131] Macrophages appear in the fetal intestine at 11–12 weeks' gestation, increase rapidly during 12–22 weeks, and then at a slower pace through early childhood.[44,132–137] These cellular groups are broadly similar in the small intestine and colon,[10] and form a critical part of the innate immune system to encounter luminal bacteria that may breach the epithelium to enter the lamina propria[112,138,139] Gut macrophages also promote peristaltic movements and promote tolerance for antigens derived from diet and commensal microbiota.

In the neonatal intestine, there are two distinct pools of macrophages, one comprised of mature macrophages that might have been derived from YS precursors, and another that could represent MDMs. During inflammation, the MDM pool enlarges and newly recruited monocytes are seen (Fig. 1). The numerical development of the macrophage pool, unlike that of mucosal lymphoid aggregates, is programmed in the fetal intestine and does not require the presence of dietary or microbial antigens.[132,140] The gut macrophage pool in the fetus contrasts with the macrophages in the lung; there are very few alveolar macrophages in the fetus and this population expands after birth.[141–152] However, the functional maturation of gut macrophages continues during early infancy and is influenced by antigens in ingested food and microbiota.

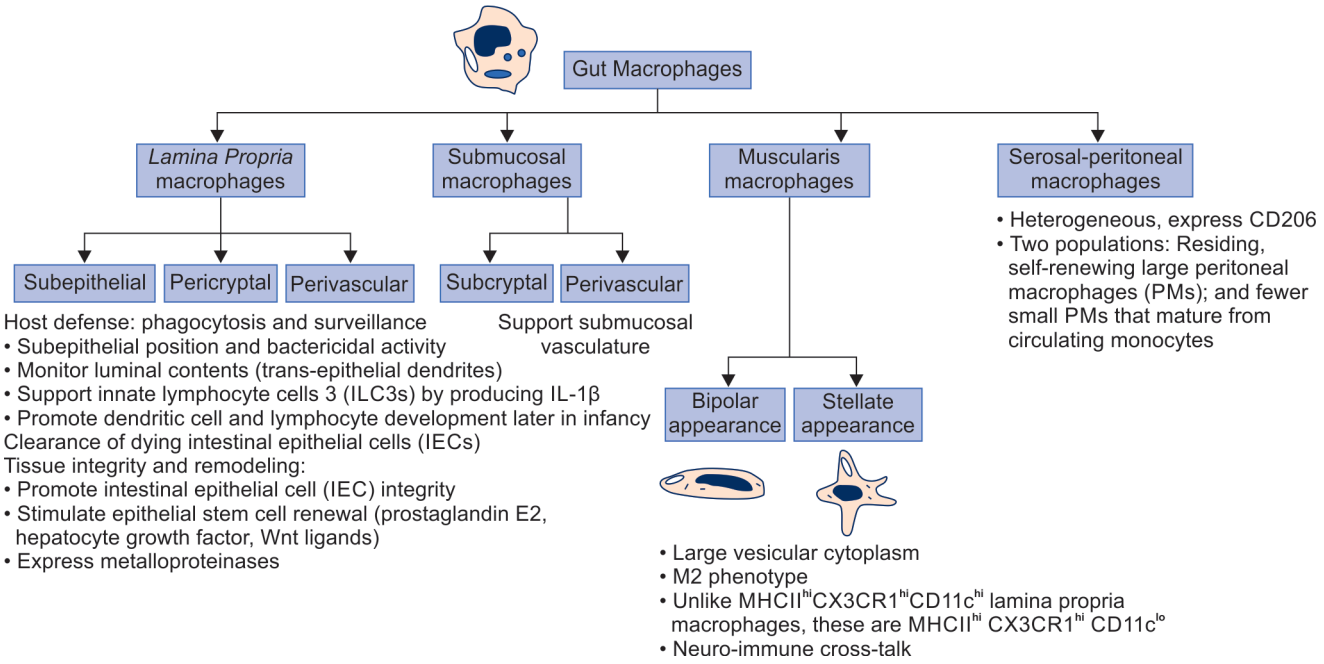
Unlike many other organs, intestinal macrophages have very limited ability to undergo clonal expansion.[138] The primary mechanism for maintaining the gut macrophage pool is through the recruitment and differentiation of blood monocytes.[138,139,153] In adults, interleukin-8/CXC ligand 8 (IL-8/CXCL8) and transforming growth factor-beta (TGF-β) recruit monocytes to the intestinal mucosa.[138] However, developmental limitations in the fetal intestine may preclude these two cytokine systems in effective recruitment of monocytes as macrophage precursors. In the fetus, IL-8 is expressed primarily as a longer, less-potent 77-amino acid isoform unlike the shorter 72-amino acid isomer in the adult.[154] Similarly, TGF-β bioactivity is low in the early-/mid-gestation fetal intestine.[155] Finally, macrophage populations begin to expand in the fetal intestine several weeks before lymphocytes or neutrophils,[132,133,135] suggesting that the recruitment of monocytes as macrophage precursors may occur via specific chemoattractant(s) other than IL-8/CXCL8, which recruits both neutrophil and macrophage precursors,[138,156] or TGF-β, which also mobilizes T-lymphocytes.[138,157]

We have reported that chemerin (the retinoic acid receptor responder-2/RARRES2) might be a key chemoattractant for



Figs 1A and B: Intestinal macrophage populations in neonatal mice. We used flow cytometry to examine intestinal lysates from mouse pups with normal intestine and from others with intestinal inflammation. Macrophages were identified as the cells expressing the myeloid marker CD11b and the macrophage marker, F4/80. The normal intestine showed two distinct pools of macrophages, one of mature macrophages that expressed F4/80 at high levels and likely represents YS-derived cells. There was a second F4/80^{mid} subset consistent with MDMs. During inflammation, the MDM pool got enlarged. Some newly recruited F4/80^{low} monocytes were also seen (expressed high levels of the monocyte marker Ly6C; not shown)

Flowchart 3: Classification of gut macrophages by location. Schematic shows the location, classification by location or shape, and the best-known function



monocytes in the normally developing fetal intestine. Chemerin is a 16 kDa heparin-binding molecule[158,159] expressed in fetal IECs beginning at 10–14 weeks with a peak at 20–32 weeks and then gradual diminution to minimal levels towards term.[160,161] The chemerin promoter contains several CpG islands located in close vicinity of retinoic acid receptor (RAR)-β binding sites;[162,163] RAR-β is now recognized as an epigenetic regulator with multiple variants, the expression of which is regulated through alternate promoter usage and differential splicing.[164] Interestingly, only monocytes, not neutrophils or lymphocytes, express the chemerin receptor, the chemokine-like receptor-1 (CMKLR1).[165,166] The developmental importance of chemerin as a monocyte chemoattractant in the intestine is also related to the fact that it is broken by cysteine proteases into fragments that inhibit the responses of inflammatory responses of these cells to bacterial products.[167] In the injured neonatal intestine, macrophage-rich infiltrates are prominent, which contrasts with the pleomorphic leukocyte infiltrates in inflammatory bowel disease or gut inflammation models in adult mice.[168–173] The chemokine CXCL5 is an important chemoattractant for macrophage precursors to the neonatal intestine.[168]

Intestinal macrophages in mammals share some common features. Unlike macrophages in other organs, human gut macrophages express CD14 and CD16 at very low levels.[174] Murine gut macrophages can be identified by the markers F4/80[175] and the fractalkine receptor CX3C receptor 1 (CX3CR1).[176] Gut macrophages typically express CD64 Fc-gamma receptor 1,[177] CD163 (scavenger receptor for free hemoglobin or the hemoglobin–haptoglobin complex),[178] and the myeloid-epithelial-reproductive tyrosine kinase (MerTK).[3] In adults, most gut macrophages except those at immune inductive sites such as Peyer's patches (PPs) typically display anti-inflammatory characteristics.[139] These properties are still maturing in neonates.[139] There are four main categories of gut macrophages (Flowchart 3).

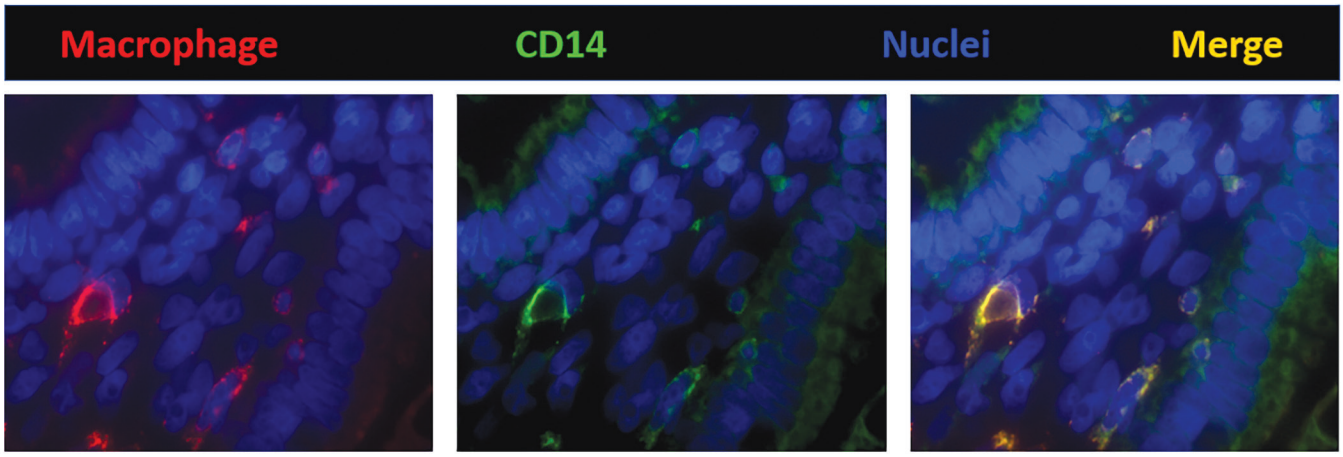


Fig. 2: Fluorescence photomicrograph (1000×) of a villus in preterm (26 weeks) human intestine shows that macrophages (HAM56, red) express CD14 (green). CD14 is an important mediator in the bacterial lipopolysaccharide (LPS)-stimulated signaling pathways. These findings are important because macrophages in the adult human intestine do not express CD14 and are unresponsive to LPS. Nuclei are stained blue (4′,6-diamidino-2-phenylindole, or DAPI, is a fluorescent stain that binds adenine–thymine-rich regions in DNA)

(a) *Lamina propria macrophages* (LPMs): These cells are located below the epithelium, around crypts, and near blood vessels in both the small intestine and the colon.[3,44,168,169,172,173] The primary functions are in host defense, clearance of dead cells, and maintenance of tissue integrity.[3] Mucosal perivascular macrophages also form tight interdigitating connections around the vasculature to prevent bacterial translocation into the blood circulation. These cells express angiogenesis-related genes necessary for the repair and strengthening of the vasculature.

LPMs can be derived from the primordial myeloid precursors developing in the intestine, those immigrating from the liver, or from the blood monocytes.[179] The maturation of Ly6c1 lymphocyte antigen 6 complex, locus C1 (Ly6C)^{hi} CC receptor 2 (CCR2)^{hi} CX3CR1^{int} monocytes into Ly6C^{lo} CCR2^{lo} CX3CRI^{hi} macrophages is driven by the microbiota and by the transcription factor nuclear receptor subfamily 4, group A, member 1 (NR4AI).[180] The fractalkine receptor CX3C receptor 1 (CX3CR1) promotes dendrite formation and luminal migration.[97] At the base of SI and colonic crypts, some LPMs that express sialoadhesion (CD169)$^{+}$ are closely associated with the stem cell niche.[181] In the small intestine, these LPMs promote Paneth cell differentiation, maintain the LGR5^{+} stem cell pool, and promote epithelial proliferation. The analogous subset in the colon is not essential to maintain the stem cell niche, but may promote regenerative responses after injury.

(b) *Submucosal macrophages*: These help maintain the submucosal vasculature. LPMs and submucosal macrophages differ in life span, transcriptional programs, and function. These cells are either self-maintaining or are derived are from regular differentiation from monocytes.[3,181] Some submucosal macrophages resemble those in the muscularis externa and are located in close proximity to the neurons and vasculature. These cells comprise a long-lived, self-maintained subset of macrophages.[181] Considering the location away from the gut lumen, this pool of macrophages does not seem to need the stimulation from the microbiota and dietary antigens for replenishment by circulating monocytes.

(c) *Muscularis macrophages* are located underneath the submucosal region between circular and longitudinal muscle layers, and are therefore, relatively distant from luminal stimuli. These cells may show either a bipolar (associated with the circular muscles and the deep muscular plexus) or a stellate (in the serosal and myenteric plexus) shape.[182] These are associated with the *muscularis externa* and associated enteric neurons, distant from any luminal stimulation.[3,183]

The development of muscularis macrophages is ensured by CSF-l produced by the enteric neurons, and possibly also by endothelial cells or interstitial cells of Cajal.[184] The gene expression profile of these macrophages suggests a role in tissue protection, neuronal development, and intestinal peristalsis. The bone morphogenetic protein (BMP) 2 and prostaglandin E2 (PGE2), which act on enteric neurons and smooth muscles, respectively, are also expressed.[182] Muscularis macrophages may also serve in a neuroprotective role by limiting infection-induced neuronal loss through the adrenergic/arginase 1/polyamines axis[185] and the norepinephrine signaling via β_2 adrenergic receptors (β_2ARs).[186]

(d) Serosal macrophages: These cells may be derived from precursors arising in the muscularis, or from peritoneal macrophages adherent to the serosa.[187] The surface markers and properties vary with the degree of inflammation in the peritoneal cavity.

Inflammatory Characteristics of Macrophages in the Developing Intestine

In the adult human intestine, newly recruited monocytes differentiating into macrophages retain avid phagocytic and bactericidal activity but develop inflammatory anergy and tolerance to bacterial products.[139] These macrophages lose innate response receptors such as CD14, Fcα (CD89), Fcγ (CD64, CD32, CD16), the integrin lymphocyte function-associated antigen 1 (LFA-1; CD11a/CD18); and the complement receptors (CR) 3 (CD11b/CD18) and CR4 (CD11c/CD18). These cells no longer produce inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-12, CC receptor ligand 5 (CCL5), and the TNF. This inflammatory downregulation occurs due to the exposure to extracellular matrix (ECM) factors such as TGF-β.

Unlike the gut macrophages in adults, those in the fetus/premature infants are yet to develop complete tolerance to bacterial products and display inflammatory responses upon stimulation. These cells express many of the inflammatory markers listed above (Fig. 2). There are three main reasons:

(1) The developing intestine has a deficiency of TGF-β, particularly its TGF-β_2 isoform.[121] During NEC, TGF-β expression and bioactivity are further reduced to levels that are even lower than in a normal fetus of a similar gestational age.
(2) Macrophages in the premature intestine are resistant to TGF-β and are therefore, intrinsically hyper-inflammatory because of high expression of an inhibitor of TGF-β signaling, the Smad7.[169] The abbreviation Smad refers to the homologies in this protein to the "*Caenorhabditis elegans*" SMA, the "small" worm phenotype and the MAD, the "Mothers against Decapentaplegicc family of genes in Drosophila. Bacterial products further induce Smad7 expression in neonatal, but not adult, gut macrophages.[169] Smad7 increases inflammatory activity by augmenting LPS-induced NF-κB activation; it activates the inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β) expression by attaching to two smad-binding elements in the IKK-β promoter, and consequent acetylation of Lys12 residue on histone 4 (H4K12).[188] Increased Smad7 expression in the developing intestine may be due to a developmental deficiency of the SKI (Sloan-Kettering Institute)-like proto-oncogene (SKIL) oncoprotein, which is a physiological repressor of the Smad7 promoter.[173,189] Our findings showed Smad7 to be an important negative regulator of TGF-β signaling in the gastrointestinal tract.[190] Smad7 can also suppress TGF-β signaling by competing with the activating Smads, increasing the degradation of the TGF-β receptors, and through epigenetic mechanisms by interacting with histone deacetylases.[191–193] IKK-β is an essential catalytic subunit of the IKK complex, which includes another catalytic subunit, IKK-α, and a regulatory subunit, IKK-γ.[194] During inflammation, cytokines and bacterial products promote phosphorylation of IKK-β, which in turn, activates the IKK complex. Activated IKK phosphorylates the IκBs, triggering their degradation and thereby releasing the NF-κB dimers for nuclear translocation. In our study, Smad7 activation of the IKK-β promoter was associated with increased H4K12 acetylation, an epigenetic marker of euchromatin,[195] on the IKK-β nucleosome. H4K12 acetylation may neutralize its electrical charge, leading to structural changes that promote DNA accessibility and interactions with the H2A–H2B dimer in neighboring histones.[196]
(3) Human milk contains substantial amounts of TGF-β, particularly the isoform TGF-β_2, but most of it is in a latent, inactive form.[197] A substantial proportion of milk-borne TGF-β_2 is also inactive because it is bound to chondroitin sulfate-containing proteoglycan(s) such as biglycan.[198]

Macrophages in immune inductive sites in the developing intestine show some unique features.[199] Peyer's patches first appear at 11 weeks and develop during mid-late gestation.[200] At birth, these lymphoid aggregates are structurally complete but "naive", as the germinal centers take a few weeks to develop. The number of PPs in the ileum increases as a function of gestational maturation, and premature infants born prior to 32 weeks' gestation may have only half as many PPs than those born at full term. Lymphoid aggregates in the vermiform appendix may develop only after birth following postnatal bacterial colonization.[200]

After birth, macrophages in PPs are exposed to more antigens than those in the *Lamina propria*.[201] These cells do not express the typical macrophage markers such as F4/80 (a glycoprotein marker seen on mature macrophages) and CD64 (IgG Fc Receptor I) in mice and CD163 (high affinity scavenger receptor for the hemoglobin–haptoglobin complex) in humans,[202] but most display the apoptotic receptor myeloid-epithelial-reproductive tyrosine kinase (MerTK) and the fractalkine receptor CX3CRI.[3] In view of high-level expression of lysozyme, these macrophages labeled as lysozyme-expressing macrophages (LysoMacs).[203] The muscularis and serosal macrophages located below the follicles express CD169 (sialoadhesin, a cell adhesion molecule), unlike those in the PP.[10] The follicular LysoMacs express the phosphatidylserine receptor T-cell membrane protein 4 (TIM-4), whereas subepithelial and upper follicular LysoMacs do not.[10] These factors could comprise one possible mechanistic explanation for the known regional specialization of macrophages inside the PPs.[204]

Macrophages outside the immune inductive sites such as in the small intestinal *Lamina propria*, both in terms of the number and functional characteristics, might be influenced by dietary factors.[205] The impact on the Janus kinases (JAK)-signal transducer and activator of transcription proteins (STAT) pathway, expression of CCL2, and inflammasome activation with increased intestinal permeability, glucose metabolism, and insulin sensitivity may be important.[206] Saturated fatty acids, derived from the metabolism of w-3 PUFAs, activate inflammatory responses through the TLR4–NF-κB pathway.[207] Some macrophages acquire properties that promote the resolution of inflammation induced by dietary antigens.[126]

Colon is functionally important for the absorption of electrolytes and water, and for the management of undigested foodstuffs.[208] In the distal colon, macrophages insert balloon-like protrusions between epithelial cells to sense their microenvironment, although these extensions do not quite extend into the lumen as seen in the small intestine.[209,210] These protrusions sample the fluids absorbed by epithelial cells to detect toxins and protect by regulating absorption of potentially toxic luminal contents.[211]

The macrophages in the colonic lamina propria are continuously exposed to anti-inflammatory microbial metabolites and short-chain fatty acids (SCFAs) such as butyrate,[212] and produce antimicrobials such as lysozyme, calprotectin, and ROS.[213] Butyrate is known to suppress inflammatory signaling by inhibiting histone deacetylase 3.[214] It also stimulates the goblet cells to produce more organized mucus by changing glycosylation-related gene expression.[215] These improved mucus layers reduce bacterial translocation and induce physico-chemical changes in the oxygen and pH levels; this feedback loop regulates the inflammatory activity by altering the number and functional diversification of macrophages.[215–218] Colonic macrophage functioning is also altered by TGF-β and IL-10 signaling. These pathways promote the interaction of Wiskott-Aldrich syndrome protein (WASP) and dedicator of cytokinesis (DOCK) 8, leading to the phosphorylation of STAT3 and the anti-inflammatory polarization of these macrophages.[219] Dectin-1 activates colonic macrophages, resulting in inflammasome-dependent IL-1β secretion and monocyte recruitment to the inflamed colon.[220]

Gut Macrophages are Key Mediators of Intestinal Inflammation in NEC

Necrotizing enterocolitis is an inflammatory bowel necrosis seen in up to 10% of very-low-birth-weight (VLBW) premature infants born prior to 32 weeks' gestation.[221] The mortality rates of NEC continue to be between 20 and 30% all over the world.[222] The pathogenesis of NEC is still unclear, but many risk factors have been identified such as chorioamnionitis, perinatal asphyxia, indomethacin therapy

in neonates, formula feedings, use of human milk fortifiers, viral infections, use of feeding thickeners, and severe anemia; anemia may either be a risk factor in itself, or may predispose to NEC following "corrective" red blood cell transfusions.[171]

Nearly 80% of all intestinal tissue specimens that have been resected for NEC showed inflammatory changes with leukocyte infiltration and edema.[223–225] Interestingly, despite the rapid progression of intestinal injury, the leukocyte populations were largely comprised of macrophages, not neutrophils.[224,226] This prominence of macrophages in NEC lesions could be a tissue-specific finding, but also possibly related to the relative immaturity of neutrophils compared to macrophages in premature infants. A few eosinophils were seen in some lesions. The number of lymphocytes did not differ between control areas and NEC lesions, although many recent reports suggest that NEC may alter lymphocyte subsets. The severity of inflammation in NEC is usually graded by the depth of changes, beginning at the mucosa and outward progression, and by the density of leukocyte infiltration. More than half of all NEC lesions show leukocyte infiltrates extending into the submucosa or beyond.

Inflammatory infiltrates can be seen in 70–80% of resected tissue samples.[168–170,172,200,226,227] These infiltrates were typically comprised of macrophages (70–80% of the leukocytes/high power microscopic field/HPF) and neutrophils (15–20% of the leukocytes/hpf).[168,224] There was a modest increase in neutrophils (37.9 ± 5.8 cells in NEC vs. 7.7 ± 1.7/high-power field in control tissues). The number of lymphocytes usually does not change significantly. A few eosinophils may be seen in the involved areas, and in some infants, inflammatory pseudomembranes and crypt abscesses can also be seen. Pender et al.[228] previously described similar inflammatory changes with macrophage infiltration with NEC. We have described how the pro-inflammatory characteristics of still-maturing intestinal macrophages in the preterm intestine can increase the risk of NEC and refer the reader to the articles listed in the references.[121,168–173,200,226,229–237] Several genetic and epigenetic factors may also alter the susceptibility to NEC and/or its severity, its clinical and histopathological manifestations, and if surgery is needed, of an adverse postoperative course and outcome.[227,238]

Conclusions

Recent years have brought considerable progress in our understanding of the heterogeneity and inflammatory immaturity of gut macrophages in premature and full-term newborn infants; particularly in the context of diseases such as NEC. There is a need for continued efforts to understand the maturation of macrophages, and the mechanisms that might derail this process, and the points where timely identification and therapeutic interventions might be of translational importance. There is hope.

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REVIEW ARTICLE

Effect of Ursodeoxycholic Acid in Unconjugated Hyperbilirubinemia in the Term Neonates Treated with Phototherapy: A Systematic Review

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Received on: 03 November 2022; *Accepted on:* 15 November 2022; *Published on:* 23 December 2022

Abstract

Background: Neonatal hyperbilirubinemia is a common clinical condition worldwide. Phototherapy (PT) is the standard intervention for hyperbilirubinemia; however, it may have side effects. It has been suggested that the implementation of adjuvant therapy including ursodeoxycholic acid (UDCA), for example, may decrease the duration of PT.

Objectives: To determine the efficacy and safety of UDCA in addition to PT in term neonates with unconjugated hyperbilirubinemia (UH) vs PT alone.

Methods: A systemic review was undertaken using the following databases: PubMed, Medline, Cochrane database, Scopus, Google Scholar, and ClinicalTrials.gov. Randomized controlled trials (RCTs) assessing the efficacy and safety of UDCA combined with PT on the total serum bilirubin (TSB) and duration of PT were included. The data quality assessment was carried out.

Results: Low–moderate quality evidence from seven RCTs reported significantly lower TSB levels in the UDCA group compared to the control group after 12, 24, 48, and 72 hours of treatment with a mean difference (MD) of −2.23 mg/dL (95% CI: from −2.49 to −1.96); −1.59 mg/dL (95% CI: from −1.83 to −1.35); −1.03 mg/dL (95% CI: from −1.27 to −0.79); and −1.32 mg/dL (95% CI: from −1.63 to −1.01), respectively, with heterogeneity of studies $I^2 = 92\%$ ($p < 0.00001$). In addition, three studies observed that UDCA significantly decreased the duration of PT with MD −19.14 hours (95% CI: from −20.70 to −17.59) with heterogeneity $I^2 = 91\%$ ($p < 0.00001$). None of the studies reported any significant adverse effects of UDCA.

Conclusion: Ursodeoxycholic acid combined with PT in the treatment of UH significantly reduces the TSB and duration of PT without significant risk of adverse events. However, limited and low–moderate quality evidence exists to support the routine use of UDCA in neonates. We discuss the limitations of the review results for clinical practice.

Keywords: Hyperbilirubinemia, Neonates, Phototherapy, Physiological jaundice, Systematic review, Ursodeoxycholic acid.

Newborn (2022): 10.5005/jp-journals-11002-0046

Introduction

Unconjugated hyperbilirubinemia is a common problem during the neonatal period and affects up to 90% of neonates.[1]

Hyperbilirubinemia becomes clinically apparent as jaundice when TSB is above 5 mg/dL (85 mmol/L) which mostly resolves spontaneously in the first weeks of life.[2] Physiologic mechanisms of neonatal jaundice (NJ) include increased breakdown of fetal erythrocytes due to the shortened life span and higher erythrocyte mass; decreased hepatic excretory capacity of bilirubin due to low concentration of the ligandin and glucoronyl transferase.[3] Neonatal UH can also happen from pathological conditions such as hemolytic disease, sepsis, polycythemia, extravasation of blood, and metabolic disorders.[4] A rise in TSB level above 20 mg/dL (>99th percentile on the hour-specific nomogram), defined as severe hyperbilirubinemia, can lead to bilirubin encephalopathy with a high risk of mortality.[5]

The prevalence of kernicterus is significantly higher (73 per 100,000 live births) in sub-Saharan Africa, and South and Central Asia compared to Europe (10 per 100,000 live births).[6] The high rate of UH in sub-Saharan Africa and South Asia regions can be correlated with maternal factors like primiparity, place of delivery, blood group incompatibility, and infant factors like prematurity, infection, glucose-6-phosphate dehydrogenase (G6PD) deficiency that can be explained by the unavailability of required intervention.[7] Furthermore, the limited financial resources, lack of effective PT equipment and traditional practice of application of dusting powder, or herbal consumption might be one of the causes of the high rate of bilirubin encephalopathy in the African region.[7–9] Currently, PT is the most widely used therapy for UH that converts unconjugated bilirubin (UCB) to water-soluble bilirubin photoproducts excreted through the bile and urine, thereby decreasing TSB level and needs for exchange transfusion.[10] However, several complications such as dehydration, diarrhea, electrolyte disbalance, and the possibility of early discontinuation of breastfeeding have been reported.[11,12] Other disadvantages include physical separation of the neonate from the mother and

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How to cite this article: Huseynova RA. Effect of Ursodeoxycholic Acid in Unconjugated Hyperbilirubinemia in the Term Neonates Treated with Phototherapy: A Systematic Review. Newborn 2022;1(4):356–367.

Source of support: Nil

Conflict of interest: None

prolonged hospital stay; all these may lead to parental emotional stress.[11,13] Another concerning PT complication is the associated risk of cancer and myeloid leukemia.[14,15] Moreover, exchange transfusion and kernicterus, despite PT, continues to be reported.[16] Hence, the concept of adjuvant intervention in addition to PT has gained increasing consideration.

An additional pharmacological product that may intensify the efficacy of PT is UDCA.[17–19] Ursodeoxycholic acid is a naturally occurring bile extract used to treat neonatal cholestasis.[20] It has low toxicity and is usually used at a pharmacological dose of 10–15 mg/kg/day. It can be administered with 1–2 mL of milk or sterile water orally or *via* an intragastric tube.[21] To date, UDCA has been reported to be relatively safe with no significant adverse effects in neonates.[22–24] The possibility of UDCA in addition to PT reducing the TSB levels may have significant potential in terms of decreasing the length of stay and associated healthcare costs.[25] That intervention maybe is a promising solution, especially in low-income countries where limited financial resources for PT equipment may be an additional factor for severe NH. However, other studies found no beneficial effect of the UDCA.[26,27] Hence, the role of UDCA on UCB levels is currently not clear.

Objectives

Primary Objectives

To determine the efficacy of UDCA in combination with PT vs PT alone on TSB levels in term neonates with UH.

Secondary Outcomes

To determine the effect of UDCA in combination with PT vs PT on the duration of PT.

To determine the frequency and nature of adverse effects of UDCA when used to treat neonates with UH.

Methods

Research Question

This review will evaluate studies assessing the effect of UDCA on reducing TSB levels in term neonates treated with PT compared to PT or PT plus placebo.

The research question is as follows: "In term neonates with unconjugated hyperbilirubinemia, does the usage of UDCA and PT compared to PT only or with placebo effectively decrease TSB levels?"

Search Strategy

An initial electronic search was conducted in 2021 using databases: Cochrane Library, Ovid MEDLINE® (1996 to week 4 of June 2021), Embase Classic + Embase (1947 to week 25 of 2021), CINAHL Plus with EBSCOhost, Medline/PubMed, DelphiS, Scopus, and Google Scholar.

The search strategy was created with keywords and subject terms by combining Medical Subject Heading (MeSH) in PubMed and Ovid. Database search techniques with usage truncation*; quotations marks to keep terms together; parenthesis and nesting to similar group terms together were applied. Search terms used were as follows: "neonate or infant or baby or newborn"; "ursodeoxycholic acid or UDCA or ursodiol or ursobil"; "hyperbilirubinemia or bilirubin or jaundice or icterus"; "PT or light therapy or PT." The final approach of combination with OR/AND Boolean operators was applied in an advanced search.

Supplemental searches were conducted in ClinicalTrials.gov and WHO's International Clinical Trials Registry (2000–2021) and did not reveal a newly resulted or completed study.

An updated search was conducted from inception to June 2021 to identify additional papers, using the same search strategy described above. An additional two articles were added; however, one article was excluded due to reported duplicated results in intervention and control groups.

The search results were limited by human, English language, newborn, and clinical trial filters. The RCT studies of neonatal hyperbilirubinemia treated with UDCA (of any dosage) in addition to PT and compared to either PT alone or placebo with PT were included. The primary outcomes evaluated were as follows: TSB after treatment, TSB decrease, duration of PT, and side effects.

Participants were well-appearing neonates with unconjugated non-hemolytic hyperbilirubinemia; age less than 28 days; gestational age above 37 weeks of gestation, weight above 2500 gm treated with PT.

Study Selection

Studies were retrieved after removing the duplicates and screening titles and abstracts. The full text was reviewed for the eligibility criteria. The resulting studies reference list was further searched for relevant articles. Finally, seven articles were selected (Flowchart 1).

Quality Assessments

Each study was subjected to critiquing with the Consolidated Standards of Reporting Trials (CONSORT) tool.[28] The review was assessed for the included studies' risk of bias (RoB) using Cochrane Collaboration's "Risk of bias" tool.[29] The following aspects were considered: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcomes' assessment, incomplete outcome data, and selective reporting, and other sources of bias. Each component of the RoB tool in the included studies was categorized as low, high, or unclear RoB. Subsequently, the quality of evidence was assessed accordingly to the GRADE tool.[30]

Data Extraction

The characteristics of the included seven studies are summarized in Tables 1 and 2.

Statistical Analysis

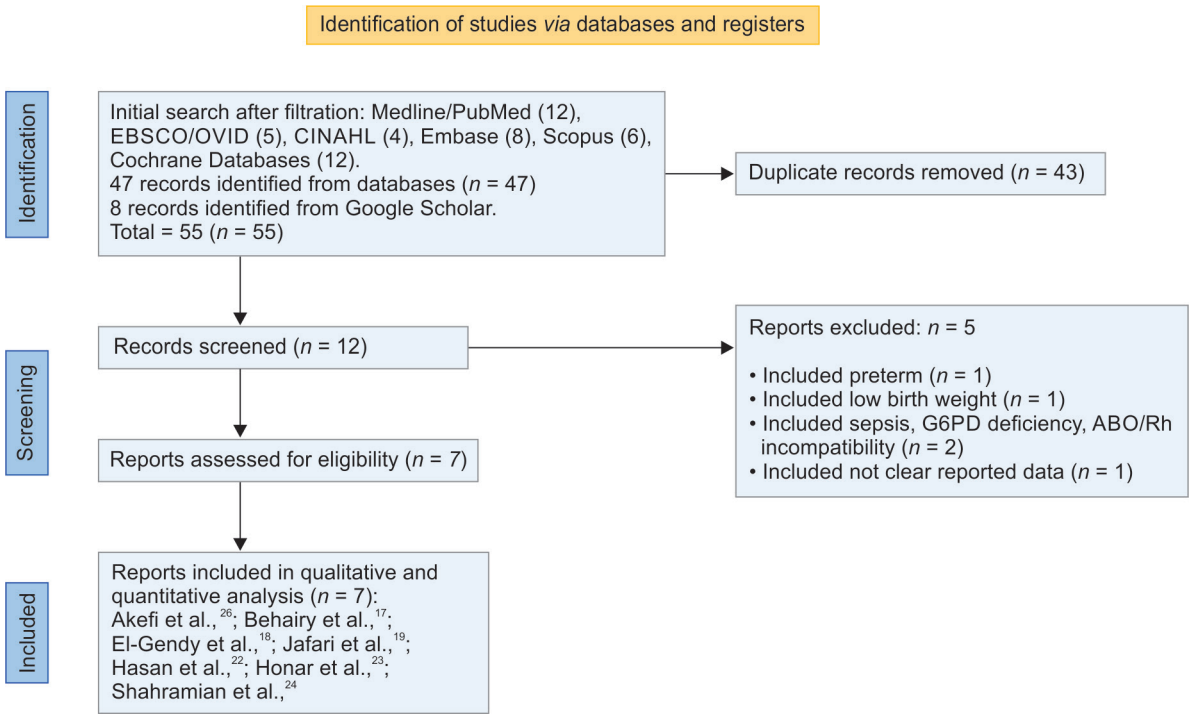
Data synthesis were carried out for the two outcomes (TSB levels and duration of PT) using the Review Manager meta-analysis software (version 5.4, The Cochrane Collaboration, 2020).

The 95% CI was calculated for each treatment group in each study. Heterogeneity among all studies was measured by the Chi-squared test and the I^2. If substantial (I^2 >50%) heterogeneity was detected, the potential causes for its presence were studied using further sensitivity analysis.

Results

The initial search found 51 studies, and those meeting the inclusion and exclusion criteria were screened. After examining the titles and abstracts of the studies found in the primary search, 12 studies were selected to read the full texts. Of these, we excluded one study which included preterm neonates,[31] two included ABO or Rh incompatibility with or without sepsis,[25,32] one due to low birth weight (<2500 gm),[33] and one study due to unclear reported data.[27]

Flowchart 1: The PRISMA Flowchart



Finally, we identified seven studies that met the inclusion criteria, they were all published in peer-reviewed journals between 2015 and 2020 and included 996 neonates.[13–15,22–24,26] All studies were reported as RCTs which according to the hierarchy of evidence are generally considered the best research design for assessing intervention effects.[34]

Four of them[14,23,24,26] were parallel double-blind studies, while the other three were open-label RCTs.[13,15,22]

Three studies were from Iran, two from Egypt, one from India, and one from Iraq. All studies were hospital-based studies and single-center, except one study[13] where the study was conducted in two centers.

All participants were term neonates, weight above 2500 gm with unconjugated non-hemolytic hyperbilirubinemia. The age ranged from 3–5 days. The sample size was calculated in the four studies although the assessed calculation of the sample size in all studies based on 95% CI, power was 80%, and 5% margin error showed sufficient sample size in all studies. Also, $p < 0.05$ was considered statistically significant in all included studies.

There was no significant difference in the baseline characteristics in the intervention and control groups in all studies.

Figures 1 and 2 summarized the RoB for each study and each RoB item as percentages across all studies.

Three studies[13,15,22] did not adequately describe the method used to generate the random sequence for participants and allocation concealment. Four of the papers[14,23,24,26] were double-blinded while the remaining three studies[13,15,22] did not report on blinding of parents, assessors, or personnel. Quality of evidence for the outcomes was assessed by GRADE tool (Fig. 3). Evidence for usage of UDCA in addition to PT in neonatal hyperbilirubinemia was weighed as very low quality mainly due to the high RoB.

Intervention and Reported Outcomes

All participants received UDCA orally at 10 mg/kg/day divided every 12 hours except the paper by Shahramian et al.[24] where the dose of UDCA was 15 mg/kg/day, and the paper by Jafari et al.[19] where 20 mg/kg/day was administered along with PT. The PT was received according to charts of the American Academy of Pediatrics in two studies,[13,26] and others did not report on the chart used for the treatment of jaundice. The timing of PT was reported as continuous in two studies,[23,24] whereas others did not comment on the timing. Two studies used light-emitting diode light therapy (LED),[13,22] two used daylight PT,[23,24] and others did not report on the type of devices. The TSB was estimated by spectrophotometry in three trials[14,22,26] and the diazo method in four studies.[13,15,23,24]

Primary Outcome

Change in Total Serum Bilirubin Levels after Initiation of Treatment with Ursodeoxycholic Acid and Phototherapy

The effect of intervention at 12, 24, 48, and 72 hours of treatment was measured with UDCA 10 mg/kg/day and one study 15 mg/kg/day (Fig. 4).

The control group included neonates treated with PT only, except Honor et al.[23] where water as a placebo in addition to PT was added.

The mean TSB was significantly lower [MD −1.53 (95% CI: from −1.66 to −1.41)]; $I^2 = 92\%$ ($p < 0.00001$) at 12, 24, 48, and 72 hours of treatment with UDCA plus PT group compared to the PT alone or PT with the placebo group.

After excluding the one study[24] that used a different dose of UDCA (15 mg/kg/day) from analysis, and outlines studies[23,26] the heterogeneity was also high $I^2 = 92\%$ ($p < 0.00001$).

Table 1: Characteristics of included studies (order by study ID)

Studies	*P-1 (Akefi et al.)*[26]	*P-2 (Behairy et al.)*[17]	*P-3 (El-Gendy et al.)*[18]	*P-4 (Jafari et al.)*[19]	*P-5 (Hassan et al.)*[22]	*P-6 (Honar et al.)*[23]	*P-7 (Shahramian et al.)*[24]
Title	The effect of UDCA on indirect hyperbilirubinemia in neonates treated with PT: a randomized clinical trial	Role of UDCA in lowering indirect hyperbilirubinemia in neonates under PT	Role of UDCA in neonates with indirect hyperbilirubinemia-an open labeled RCT	Role of UDCA in neonates with indirect hyperbilirubinemia-an open labeled RCT	Effect of UDCA in lowering neonatal indirect hyperbilirubinemia: An RCT	Effect of UDCA on indirect hyperbilirubinemia in neonates treated with PT: A randomized trial	Therapeutic effects of UDCA in neonatal indirect hyperbilirubinemia: A randomized double-blind clinical trial
Country	Iran	Egypt	Egypt	India	Iraq	Iran	Iran
Sample size	220	100	100	96	200	80	200
Methods	RCT	RCT	RCT	RCT	RCT	RCT	RCT
Aim of study	Investigate the effect of UDCA in neonates with UH treated with PT	Aim of this study was to assess the additive effect of UDCA in reducing indirect hyperbilirubinemia in neonates under PT	Evaluate the effect of UDCA on neonatal hyperbilirubinemia treated with PT	To assess the fall of TSB rate in neonates with indirect hyperbilirubinemia treated with PT and UDCA; Compare 10 mg/kg/day vs 20 mg/kg/day of UDCA in treating UH; Study duration of PT in both groups	Evaluate the additive effect of UDCA on reducing UH in neonates treated with PT	Evaluate the effect of UDCA on reducing UH in neonates treated with PT	To investigate the synergistic effect of UDCA in combination with PT in treating indirect neonatal hyperbilirubinemia
The participants	Gestational age of 37–41 weeks with non-hemolytic jaundice, age >48 hours; weight:2500–4000 gm	Term with the onset of indirect hyperbilirubinemia from the second day of life	Neonates with indirect hyperbilirubinemia, age ≥3 days old,	Term neonates, age >24 hour, up to 14 days old	Term, with weight appropriate for gestational age, age 3–7 days old	Gestational age of 38–41 weeks, age >3 days old, weight 2.5–4 kg	Gestational age of 38-41 weeks, age of 3–5 days old, weight: 2.5–4 kg
Inclusion criteria	TSB 14–20 direct bilirubin <1 mg/dL	TSB level of 14–20 mg/dL	TSB level of 14–20 mg/dL	TSB level in PT zone	TSB level of 14–20 mg/dL direct bilirubin level <2 mg/dL	TSB level:14–20 mg/dL, direct bilirubin level <2 mg/dL	TSB level:12–22 mg/dL, direct bilirubin level <2 mg/dL
	Breast milk fed			Direct bilirubin level <20% of total	Exclusive breast milk fed	Exclusively breast fed	Exclusively breast fed
Exclusion criteria	Preterm neonates, neonates with septicemia, ABO or Rh incompatibility, diseases resulting in indirect hyperbilirubinemia (Crigler–Najjar syndrome, Gilbert); hypothyroidism; neonates with hemolysis or G6PD deficiency; infants with low hemoglobin levels Weight loss more than 10% of weight compared to birth weight on admission.	Infants with Rh or ABO incompatibility, infants with TSB level > 20 mg/dL; premature neonates; infants with neonatal sepsis	Premature neonates, neonates with severe hemolysis; sepsis; cholestasis	Infants with TSB level on exchange transfusion or who was previously exposed to PT; infants with Rh or ABO incompatibility; any congenital anomalies; sepsis	Premature neonates; Infants with Rh or ABO incompatibility; Sepsis; infants of diabetic mothers	Premature neonates; infants with ABO or Rh incompatibility; G6PD deficiency; direct hyperbilirubinemia; sepsis; diseases leading to hyperbilirubinemia (Crigler–Najjar syndrome, Gilbert syndrome, hypothyroidism/hyperthyroidism, liver diseases, etc.); infants of diabetic mothers	Premature neonates infants with ABO or Rh incompatibility; G6PD deficiency; direct hyperbilirubinemia, septicemia; and diseases leading to hyperbilirubinemia (Crigler–Najjar syndrome, Gilbert syndrome, hypothyroidism/hyperthyroidism, and liver diseases); infants with diabetic mothers

(Contd...)

Table 1: *(Contd...)*

Studies	*P-1 (Akefi et al.)*[26]	*P-2 (Behairy et al.)*[17]	*P-3 (El-Gendy et al.)*[18]	*P-4 (Jafari et al.)*[19]	*P-5 (Hassan et al.)*[22]	*P-6 (Honar et al.)*[23]	*P-7 (Shahramian et al.)*[24]
Intervention	Orally UDCA 10 mg/kg/day every 12 hours with diluted water. For the high TSB based on the standard curve, intensive PT was applied and if TSB was <3 mg/dL less than intensive PT, simple PT was applied. The mean duration of PT was measured in both groups. Duration of PT continued until TSB level <12 mg/dL Discharge criteria was based on a standard curve (Lissauer et al.), that was usually bilirubin level at the time of discharge is half of the exchange bilirubin level. If the neonate does not have a risk factor, and is >4 days old, the neonate was discharged with TSB <12 mg/dL	Orally UDCA 10 mg/kg/day every 12 hours along with PT. TSB and direct bilirubin were measured on admission and followed up every 12 hours till TSB became >10 mg/dL	Orally UDCA at 10 mg/kg/day orally divided every 12 hours along with single PT. The mean TSB measured in both groups every 24 hours till PT discontinued (24, 48, 72, and 96 hours). The mean duration of PT was measured in both groups.	Group II received orally UDCA at 10 mg/kg/day and group III received 20 mg/kg/day orally divided every 12 hours along with PT UDCA was orally administered every 12 hours 12 along with PT in groups B and C Total serum bilirubin levels were measured after 8, 16, 24, 36 and 48 hours and every 12 hours thereafter till the TSB was normalized	Orally UDCA at 10 mg/kg/day orally divided every 12 hours along with PT. TSB levels were measured every 12 hours until reaching below 10 mg/dL and then PT was stopped	Orally UDCA at 10 mg/kg/day orally divided every 12 hours along with PT. In addition, total bilirubin levels were measured by diazo method, every 12 hours until the total bilirubin level reached <10 mg/dL when PT was stopped	Orally UDCA at 15 mg/kg UDCA daily divided 12 hours along with PT. TSB and direct bilirubin levels were measured every 24 hours until the total bilirubin level reached <10 mg/dL when PT was stopped
Comparator	Only PT	Only PT	Only PT	Only PT	Only PT	PT plus placebo (water)	Only PT
Measured outcomes	(1) TSB level (mg/dL) at different time points in both groups (2) Duration of PT in both groups (hours)	(1) TSB level (mg/dL) at different time points in both groups (2) Duration of PT in both groups (hours) (3) Direct bilirubin level	(1) TSB level (mg/dL) at different time points in both groups (2) Duration of PT in both groups (hours)	(1) TSB level at different time points in all groups (2) Duration of PT in all groups (3) Compare rate of fall of TSB level in the group receiving 10 mg vs 20 mg/kg/day	(1) TSB level at different time points in both groups (2) Duration of PT in both groups	(1) TSB level at different time points in both groups (2) Duration of PT in both groups	(1) TSB level at different time points in both groups (2) Direct bilirubin level at different time points in both groups

Outcomes							
Adding USDA to PT in treatment of neonatal UH enhances TSB decrease after 24 hours of treatment ($p = 0.001$), however, no effect on the duration of PT and hospitalization time ($p = 0.63$). That result does not support UDCA treatment for UH	Adding USDA to PT in treatment of neonatal UH enhances TSB decrease ($p < 0.05$). The mean duration under PT till reaching TSB <10 was significantly lower than that in group ($p < 0.0001$). UDCA is considered an effective and safe complementary therapeutic adjuvant in neonatal indirect hyperbilirubinemia.	Adding USDA to PT in treatment of neonatal UH is highly effective; the duration of PT and hospitalization time will be shorter ($p < 0.001$)	Adding USDA to PT in neonates with UH leads to faster resolution of hyperbilirubinemia during the first 48 hours and also reduce the duration of PT	Mean of TSB was statistically lower at 12, 24, and 36 hours after hospitalization in the intervention group of neonates that received UDCA in addition to PT ($p = 0.001$)	Mean of TSB was statistically lower in intervention group during the first 48 hours of hospitalization ($p < 0.05$) Mean time required for PT decrease significantly in intervention group ($p = 0.001$)	UDCA in combination with PT can reduce the total bilirubin among neonates with UH

Mean Total Serum Bilirubin after 12 Hours after Initiation of Treatment

Four studies[13,22,23,26] contributed to this analysis. There was significantly lower TSB in the UDCA plus PT group compared to the PT alone or PT with the placebo group (MD −2.23 mg/dL; 95% CI: from −2.49 to −1.96). Heterogeneity was high $I^2 = 95\%$ ($p < 0.00001$).

Mean Total Serum Bilirubin after 24 Hours after Initiation of Treatment

Seven studies[13–15,22–24,26] contributed to this analysis showed significantly lower TSB in the UDCA plus PT group compared to the PT alone or PT with the placebo group (MD −1.59 mg/dL; 95% CI: from −1.83 to −1,35). Heterogeneity was high $I^2 = 91\%$ ($p < 0.00001$).

Mean Total Serum Bilirubin after 48 Hours after Initiation of Treatment

Four studies[13,14,23,24] contributed to this analysis showed significant lower TSB in the UDCA plus PT group compared to the PT alone or PT with the placebo group (MD −1.03; 95% CI: from −1.27 to −0.79). Heterogeneity was high $I^2 = 92\%$ ($p < 0.00001$).

Mean Total Serum Bilirubin after 72 Hours after Initiation of Treatment

Two studies[14,24] contributed to this analysis showed significantly lower TSB in the UDCA plus PT group compared to the PT alone or PT with the placebo group (MD −1.32; 95% CI: from −1.63 to −1.01). There was no heterogeneity $I^2 = 0\%$ ($p < 0.00001$).

Secondary Outcome

Mean Duration of Phototherapy (Hours) after Initiation of Treatment with Ursodeoxycholic Acid and Phototherapy

Three studies[14,22,23] contributed to this analysis (Fig. 5). There was a significantly lower duration of PT in the UDCA plus PT compared to the PT alone or PT plus placebo group (MD −19.14 hours; 95% CI: from −20.70 to −17.59). Heterogeneity was high $I^2 = 91\%$ ($p < 0.00001$).

The Adverse Effect of Ursodeoxycholic Acid

All seven studies reported no adverse effect of UDCA (irrespective of dose). In addition, none of the studies reported neonatal mortality, exchange transfusion, or bilirubin encephalopathy.

Discussion

This review identified that UDCA in combination with PT was associated with the decreased total bilirubin level in the first 72 hours of treatment. In addition, the data showed a reduction of TSB was more significant in the first 12 hours after the treatment with UDCA, suggesting that early treatment with UDCA may be more beneficial in reducing TSB levels.

Although, another systematic review conducted by Kuitunen et al.[35] showed that usage of the UDCA as adjuvant to PT resulted in shorter PT duration and faster TSB level decline, although, the concerns were related to the high heterogeneities among the included studies. Instead, a recent meta-analysis by Lazarus et al. concluded that UDCA with PT was effective in reducing the Pt duration and reducing the TSB levels.[36]

There are several hypotheses related to how UDCA may reduce the UCB. For example, Cuperus et al.[37] suggest the reducing effect of UDCA on TSB is influenced by UDCA's inhibitory action

Table 2: Characteristics of included studies (order by study ID)

				Mean TSB at different time points (hours)				
Papers	*Group*	*Age (days)*	*BW (gm)*	*Baseline*	*12*	*24*	*48*	*72*
P-1 (Akefi et al.)[26]	Intervention	4.9 ± 21.0	N/A	16.85 ± 2.4	13.14 ± 2.3	10.82 ± 2.1	N/A	N/A
	Control	5.3 ± 2.9	N/A	15.75 ± 2.6	13.05 ± 2.5	10.97 ± 2.3	N/A	N/A
P-2 (Behairy et al.)[17]	Intervention	5.48 ± 1.09	3.28 ± 0.36	17.20 ± 1.26	13.82 ± 1.11	11.94 ± 1.60	9.48 ± 1.33	N/A
	Control	5.01 ± 1.30	3.19 ± 0.37	16.91 ± 1.35	15.15 ± 1.41	13.70 ± 1.25	11.47 ± 1.13	N/A
P-3 (El-Gendy et al.)[18]	Intervention	4.90 ± 1.44	N/A	16.5 ± 1.51	N/A	13.6 ± 1.47	10.9 ± 1.19	9.13 ± 0.74
	Control	4.86 ± 1.60	N/A	16.4 ± 1.57	N/A	14.5 ± 1.63	12.2 ± 2.34	10.5 ± 1.35
P-4 (Jafari et al.)[19]	Intervention	N/A	N/A	16.22 ± 1.67	N/A	13 ± 0.0	N/A	N/A
	Control	N/A	N/A	16.47 ± 1.65	N/A	15.32 ± 1.72	N/A	N/A
P-5 (Hassan et al.)[22]	Intervention	5.4 ± 1.4	3.2 ± 0.4	16.3 ± 1.7	11.7 ± 1.5	8.8 ± 1.1	N/A	N/A
	Control	5.3 ± 1.5	3.1 ± 0.4	16.5 ± 2.9	14.6 ± 1.6	13.2 ± 5.8	9.1 ± 0.8	N/A
P-6 (Honar et al., 2016)[23]	Intervention	3.7 ± 1	2.97 ± 0.3	15.9 ± 1.7	12 ± 1.6	10 ± 1.1	9.8 ± 0.2	N/A
	Control	3.6 ± 1	2.98 ± 0.3	16.3 ± 1.5	14.4 ± 1.3	12.5 ± 1.4	10.1 ± 1.1	N/A
P-7 (Shahramian et al.)[24]	Intervention	3–5	N/A	15.79 ± 2.18	N/A	12.77 ± 1.86	10.08 ± 1.66	8.94 ± 1.38
	Control	3–5	N/A	16.89 ± 2.49	N/A	14.28 ± 2.05	11.62 ± 2.46	10.26 ± 1.92

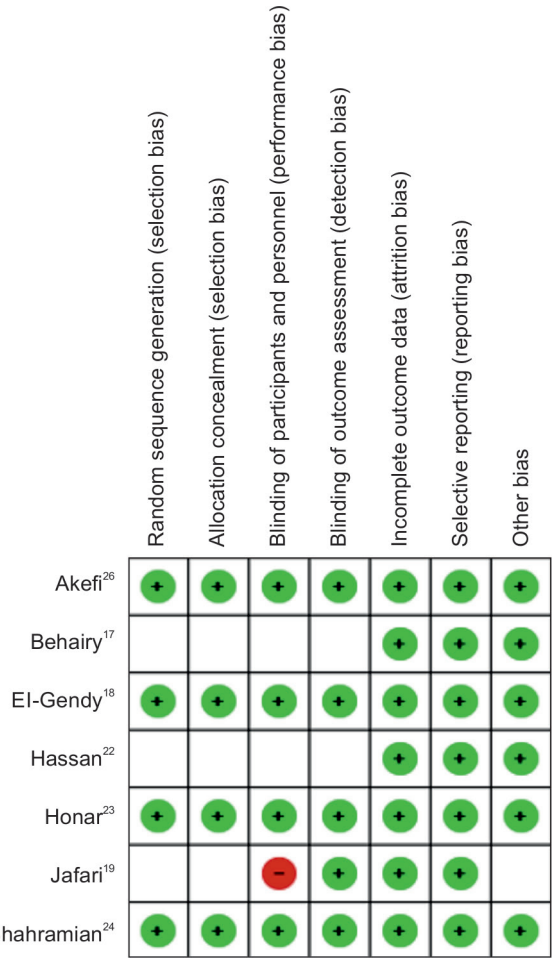


Fig. 1: Risk of bias for each included study

on the enterohepatic recirculation of UCB and increased fecal secretion. In addition, it has been reported that UDCA may improve gastrointestinal motility, which may further increase UCB turnover and decreased enterohepatic circulation.[38] The possibility that UDCA inhibits β-glucuronidase is another factor that could explain the effect of UDCA on the TSB level.[4] The reducing effect of UDCA on TSB could also be attributed to its ability to protect cholangiocytes from the cytotoxic effect of hydrophobic bile acids and improve the secretory function of hepatocytes.[39]

Furthermore, this review identified that adding UDCA to PT in neonatal UH may decrease the duration of PT by 19.14 hours (95% CI: from −20.70 to −17.59).

However, the evidence was of low–moderate quality that affected by the generalizability as some studies were found with a small number of participants and a high RoB. Interestingly, that Akefi et al.[26] observed that the effect of the UDCA was more prominent in the first 12 hours of treatment, however, without significant impact on the duration of PT and hospital stay ($p = 0.63$). The author described the usage of intensive or simple PT according to the standard curve for TSB levels. However, it was not clear how many neonates were on intensive PT and for how long. Alizadeh et al.[40] observed that intensive PT (16 lamps) declined the TSB level more than double PT (8 lamps) mainly in the first six hours of treatment compared to the second six hours of PT. A recently conducted systematic review demonstrated a significant decline in TSB levels in neonates treated with double PT compared with a single at 24 hours of PT.[41]

It may suggest that intensity of PT may further influence the effect of UDCA on UH.

Besides, breastfeeding as an inclusion criterion was mentioned in the four studies.[22–24,26] There is a significant association between NJ and breast milk leading to breastfeeding jaundice and breast milk jaundice; both types result in increased enterohepatic circulation.[42] Further, breastfeeding may lead to the interruption of PT that could delay the therapeutic effect of UDCA and PT.

However, a recent systematic review concluded that both intermittent and continuous PT appeared to be equally effective for treating UH.[43]

El-Sakka et al.[44] reported that although PT effectively decreased UCB levels in breastfed neonates, these neonates demonstrate a slower response to PT than a bottle or mixed-fed neonates.

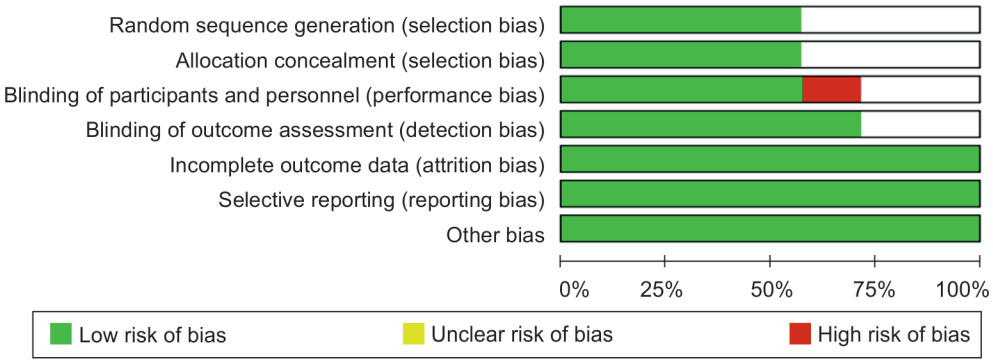


Fig. 2: Risk of bias item as percentages across all studies

However, the result of the studies with breastfed participants in our research showed a similar reduction of TSB levels as others studies. That could support the combined reducing effect of UDCA with PT at the TSB.

Moreover, the synergistic effect of UDCA with PT can be explained by the fact that unstable bilirubin photo-isomer formed under PT mechanism excreted in the intestine may further revert and be reabsorbed *via* enterohepatic circulation, increasing the plasma bilirubin level.[45] In contrast, UDCA may decrease enterohepatic circulation, increase the availability of UCB in the intestine for further metabolism by intestinal flora and fecal excretion leading to a reduction of UCB level.

In addition, the effectiveness of PT at declining TSB levels is determined by the dose of PT provided to neonates. Therefore, any changes in distance applied between the light and neonate's skin, spectral power (irradiance level), and surface body area could significantly influence the effectiveness of PT and, subsequently, the outcomes.[46] However, we found limited data about the main characteristics of PT devices in included studies that could be a reason for high heterogeneity among the studies $I^2 = 92\%$ ($p < 0.00001$).

Overall, the RoB of the included studies was high. The knowledge of treatment allocation may lead to bias like selection, detection, and performance bias that affect the study's internal validity, while non-blinded studies might increase the risk of performance bias.

Water used as a placebo study conducted by Honar et al.[23] which most probably was not as similar in appearance as UDCA capsules, made the process of blindness questionable with a high risk of performance bias. Furthermore, there was limited data about the placebo characteristics, like if the amount of the placebo used was similar to UDCA or not; if UDCA and placebo were distributed in a similar-looking container and labeled with the code or not.

Additionally, studies also varied in research methods, sample size, and duration of intervention. Besides, the usage of the different methods for bilirubin determination also could increase the heterogeneity between the studies.

Several studies revealed a difference in the results of bilirubin with the usage of different methods.[47,48] Similarly, Apperloo et al.[49] observed up to 22% discrepancy between spectrophotometric and diazo methods. Hence, different methods for TSB determination may lead to inconsistent clinical decision.

Furthermore, different doses of UDCA used in the studies may lead to high heterogeneity among the studies. The dose of UDCA varied between 10–20 mg/kg/day.[15,24]

However, removing the study with usage UDCA 15 mg/kg/day from the analysis did not affect heterogeneity. In addition, Jafari et al.[19] did not find a significant difference in the outcomes compared to UDCA at 10 mg/kg/day vs 20 mg/kg/day; both doses effectively reduced UH.

Ursodeoxycholic acid is often used off-label for hepatic or biliary disorders in pediatrics, with the most frequently reported adverse effect being diarrhea.[50] Although there are concerns associated with the risk of the development of hepatic injury from the possible accumulation of the lithocholic acid metabolite.[51] However, the study conducted in preterm neonates demonstrated a significantly lower level of γ-glutamyl transpeptidase after treatment of UDCA.[52,53]

Although the review identified the beneficial effect of UDCA in combination with PT for the treatment of neonatal UH, however, the evidence was low–moderate quality, mainly because of no proper or unclear randomization methods, allocation concealment, and non-blinding approaches. Although trial settings and study populations were comparable in the studies, the findings of the trials are generalizable; however, the impression affected the generalizability was studied with a small sample size and RoB.

Notably, it may further predispose to high heterogeneity between the studies.

Indirectly Supported Data

The additive effect of UDCA on reducing TSB was supported in preterm neonates and neonates with low birth weight.[31,33] However, Gharehbaghi et al.[31] state that UDCA at dose 15 mg/kg/day was more effective than UDCA at 10 mg/kg/day in reducing TSB levels. Furthermore, the participants in a trial of Gharehbaghi et al.[31] were preterm neonates above 35 weeks of gestation with a mean TSB before intervention slightly higher (19.6 mg/dL) compared to the mean TSB of 16.4 mg/dL of term neonates in our study.

Based on these data, we may suggest that gestational age, the onset of intervention, and TSB level may influence the effect of UDCA on UH in neonates treated under PT.

Moreover, Ughasoro et al.[25] concluded that UDCA combined with PT is also beneficial in neonates with ABO incompatibility and sepsis as decreases TSB level and PT duration ($p = 0.001$).

However, it should be noticed that UDCA is not licensed for children, and usage of UDCA in neonates is based mainly on the not well-structured reports and theoretical points.[54]

The lack of evidence from the well-structured randomized double-blind trials may suggest that the safety of UDCA usage for neonatal hyperbilirubinemia at present remains unproven.

Ursodeoxycholic acid (UDCA) Versus No UDCA for

Patient or population: patients with
Settings:
Intervention: Ursodeoxycholic acid (UDCA) Versus No UDCA

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	**Ursodeoxycholic acid (UDCA) Versus No UDCA**				
Mean Total Serum Billirubin (TSB) at different time in hours		The mean mean total serum billirubin (tsb) at different time in hours in the intervention groups was **1.65 lower** (1.79 to 1.5 lower)		2104 (6 studies)	⊕⊖⊖⊖ **very low**[1,2,3]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] High risk of bias for 1 study (Jafari 2018 & unclear for allocation & blinding in 3 studies
[2] I square >90%
[3] The result is not clinically significance

Ursodeoxycholic acid (UDCA) Versus No UDCA for

Patient or population: patients with
Settings:
Intervention: Ursodeoxycholic acid (UDCA) Versus No UDCA

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	**Ursodeoxycholic acid (UDCA) Versus No UDCA**				
Duration of Phototherapy		The mean duration of phototherapy in the intervention groups was **19.14 lower** (20.7 to 17.59 lower)		500 (3 studies)	⊕⊕⊖⊖ **low**[1,2]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Unclear allocation & blinding in one study (Hassan 2015)
[2] I square is 91% but all studies are supporting UCDA

Fig. 3: Quality of evidence assessed by GRADE tool

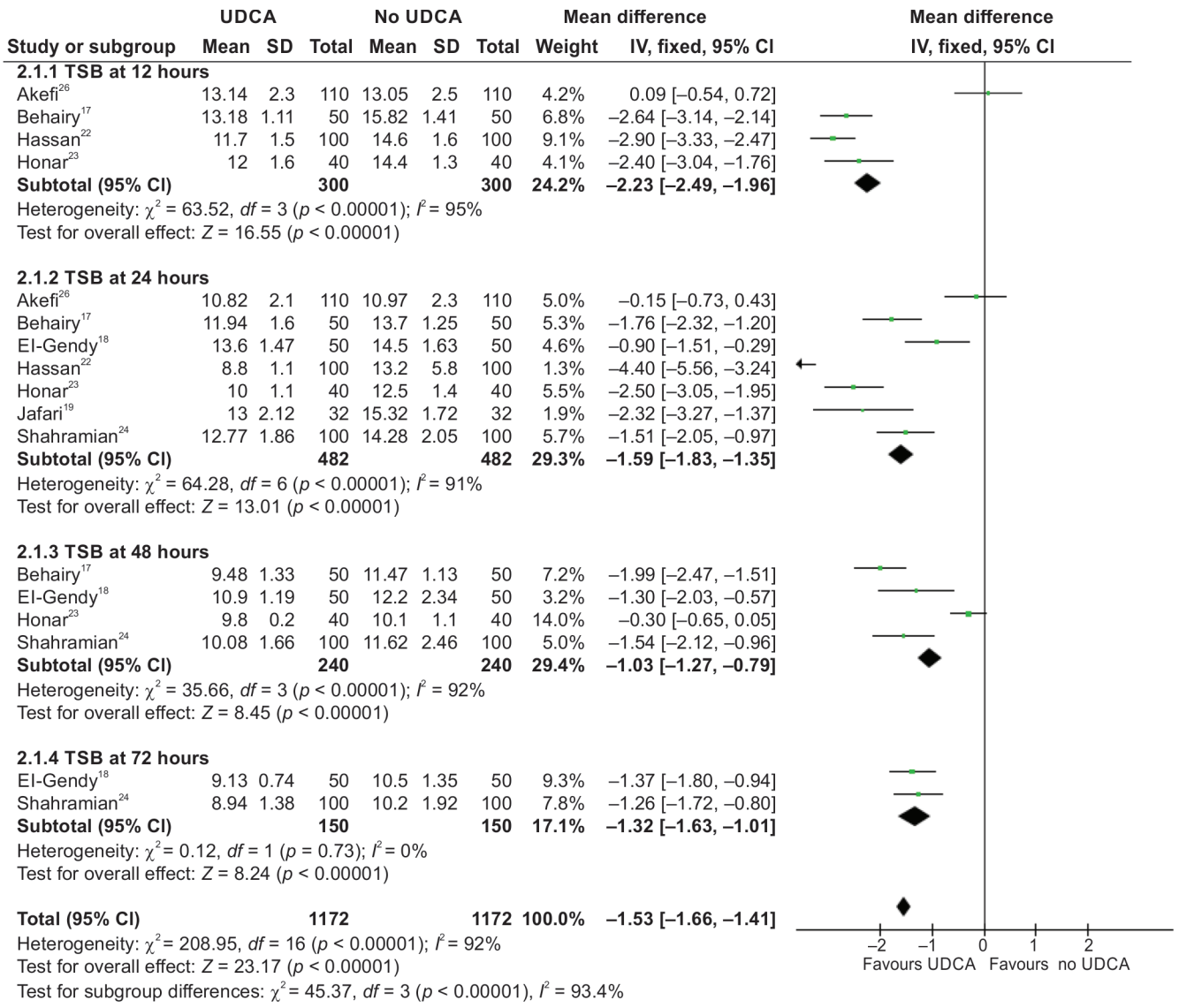


Fig 4: Ursodeoxycholic acid combined with PT vs PT alone or PT plus placebo in reducing of TSB levels

Limitations

The overall heterogeneity in study designs, the small size of studies, the lack of follow-up to monitor the rebound bilirubin levels, and any side effects of UDCA were the main limiting factors. Whether the primary response is a good predictor of long-term advantage is unknown. All included studies were conducted in limited geographical zones (Iran, Egypt, Iraq, and India). Studies involving more geographic areas are needed to be conducted prior to the standard adding UDCA to PT.

In most of the studies, the RoB was high. Furthermore, both intervention drug and comprehensive device descriptions were not described in detail. The reliability of TSB measurement methods was not satisfactory due to the variability of methodologies used in the trials.

In addition, our review was limited to term neonates with indirect physiologic hyperbilirubinemia; the mean reduction of direct bilirubin during different time points was not measured except for one trial.

Finally, since the effect of UDCA may be related to increase UCB turnover through its stool disposal and increase gastrointestinal motility, measurement of the frequency of the stool may further assess the role of UDCA in reducing TSB levels that were not analyzed.

Conclusions

Our study revealed that UDCA combined with PT reduces TSB levels in the first 72 hours with maximum effectiveness in the first 12 hours of treatment. Furthermore, the addition of UDCA to PT led to at least 19 hours reduction of PT in neonates with UH, however, the evidence was limited and low–moderate quality. Besides, all studies reported no side effects during the study period, this study does not support the usage of the UDCA in neonates at present as the safety of the UDCA in neonates is not evidence-based. It will be advisable to have long-term follow-up double-blind studies involving a higher number of participants to determine the incidence of any long-term complications and safety.

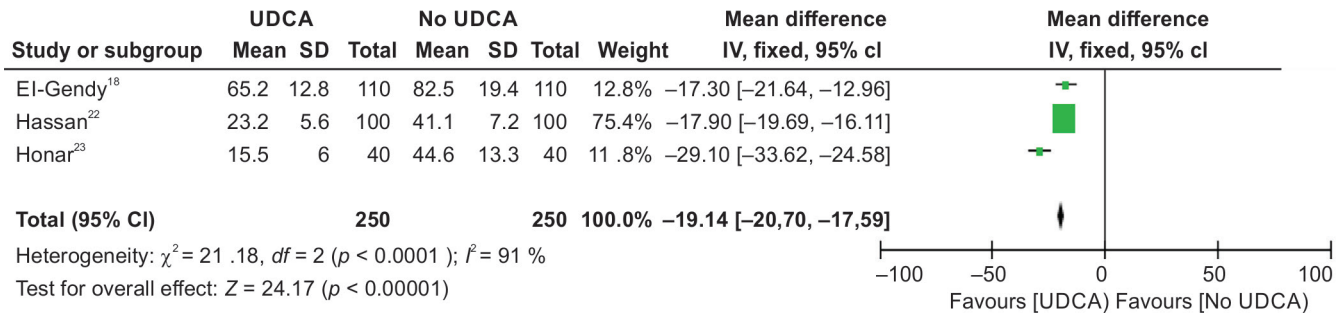


Fig. 5: Duration of phototherapy (in hours) in group with UDCA combined with PT vs PT alone or PT plus placebo group

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REVIEW ARTICLE

Hepatitis B Infections in Neonates

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Received on: 23 November 2022; *Accepted on:* 25 November 2022; *Published on:* 23 December 2022

Abstract

Hepatitis B infections are estimated to affect more than 2 billion people worldwide. The overall prevalence of HBsAg positivity in plasma is reported to be 3.5%, but it varies depending on the geographic area. Mother-to-child infection is the predominant mode of transmission in high-prevalence areas. In exposed infants, universal hepatitis B vaccination and the administration of hepatitis B immunoglobulin (HBIg) within 12 hours following the birth can reduce the risk of perinatal infection. The rates of progression to chronic hepatitis B infection depend on the age of infection and are the highest in perinatally acquired infections, thus underscoring the importance of measures to reduce transmission. Timely identification and treatment of the affected pregnant women and immunoprophylaxis of newborn infants are of paramount importance to reduce the burden of chronic infection.

Keywords: Epidemiology, Hepatitis B, Maternal hepatitis B infection, Maternal-to-neonate transmission, Neonate, Prevention.

Newborn (2022): 10.5005/jp-journals-11002-0049

Highlights

- Timely detection of maternal hepatitis B infection and antiviral treatment, in addition to the treatment of newborn infants with vaccination and administration of HBIg can reduce perinatal transmission by nearly 95%.
- The administration of tenofovir alafenamide fumarate (TAF) in pregnant mothers with high viral loads can reduce mother-to-child transmission of the hepatitis B virus (HBV).
- The recommended 3-dose vaccination series after the administration of a dose at birth should be completed by 24 weeks of postnatal age.
- Postvaccination anti-hepatitis B surface (anti-HBS) antibody titers that are more than or equal to 10 mIU/mL are seroprotective.
- Neonates with hepatitis B infections are mostly asymptomatic and need close follow-up and monitoring of liver enzymes and serology.
- Revaccination with the second series of hepatitis B vaccines for neonates who are hepatitis B surface antigens (HBsAg) negative but have anti-HBs antibodies less than 10 mIU/mL should be considered as per new Centers for Disease Control and Prevention (CDC) recommendations for the prevention of hepatitis B infection.

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How to cite this article: Anand P, Singh S, Schelonka RL, *et al*. Hepatitis B Infections in Neonates. Newborn 2022;1(4):368–375.

Source of support: Nil

Conflict of interest: None

Introduction

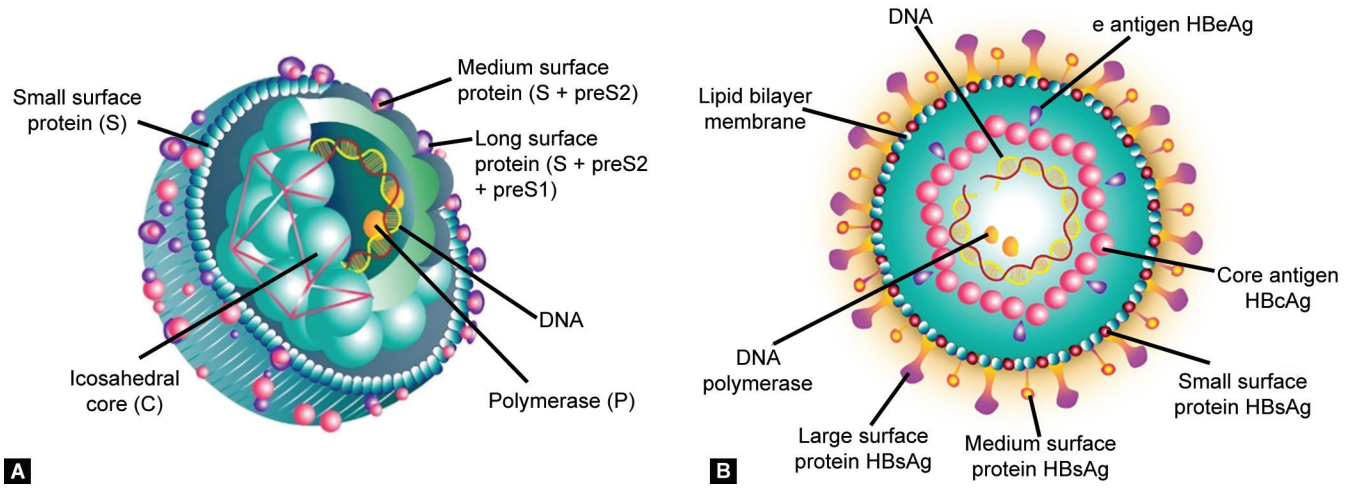
Hepatitis B is a global health problem and approximately 2 billion people worldwide have evidence of present or past Hepatitis B infection. Although HBsAg can be detected in up to 3.5% of infants worldwide, the incidence varies depending upon the geographical region.[1,2] The global prevalence of hepatitis B infections ranges between 4.2% and 6%.[3] In high endemicity regions such as in parts of China, the incidence can approach 7%.[4] Similarly, the incidence in India is estimated to be 3–4% with 40 million carriers.[5] This substantial variation is mainly related to differences in the age at infection, which is in turn inversely related to the risk of chronicity.[6]

The prevalence in children below 5 years of age reflects the effectiveness of hepatitis B vaccination coverage.[7] Latest estimates put the global prevalence figures to be less than 1%. The prevalence of chronic HBV infection in pregnant women is shown to be 0.8% but the risk of vertical mother-to-child transmission remains concerning.[8]

Structure

Hepatitis B virus is a small, double-stranded DNA virus[9] (Fig. 1). It resembles retroviruses in many aspects as it replicates through an RNA intermediate and can integrate into the host genome, which enables it to persist in infected cells.[10] Electron microscopy shows three types of viral-associated particles; the virion, spherical subviral particles (SVPs), and filamentous SVPs.[11]

The small subviral envelope spherules typically measure about 20-nm diameter. The filaments may be up to 22-nm long, contain the HBsAg and host-derived lipids without viral nucleic acids, and are non-infectious.[11] The fully-formed infectious HBV virions, known as the Dane particles, are enveloped, spherical, double-shelled structures that measure about 42-nm diameter.[12] The lipid envelope contains HBsAg, and encloses an inner nucleocapsid



Figs 1A and B: Structure of HBV. (A) Geometric structure of the spherical virion; (B) Major chemical components of the virion

comprised of the hepatitis B core antigen (HBcAg), polymerase, and the DNA genome. Hepatitis B e-antigen (HBeAg) is a HBV protein, produced by the HBcAg reading frame; it is an indicator of active viral replication. The viral polymerase is covalently attached to the 5′ end of the minus strand.[13] HBV has a partially double-stranded circular DNA genome of about 3.2 kilobase (kb) pairs with four overlapping open reading frames (ORFs: *S*, *C*, *P*, and *X*).[14]

The *S* ORF is subdivided into the pre-S1, pre-S2, and S regions and encodes three viral envelope proteins- large (L-), middle (M-), and small (S-) surface antigen (HBsAg).[15] The core or *C* gene has the pre-core and core regions and *C* ORF encodes the HBcAg or HBeAg depending on initiation of translation. The core protein forms the capsid structure while the pre-core ORF codes for a signal peptide which helps in secretion of HBeAg.[16]

The *P* ORF is the longest and encodes the polymerase (pol), a large protein comprised of the following three domains: The terminal protein domain, responsible for encapsidation and initiation of minus-strand synthesis; the reverse transcriptase (RT) domain, for genome synthesis; and the ribonuclease H domain, for facilitating replication by degradation of pre-genomic RNA (pgRNA).[17] After entry of the viral genome into the host nucleus, the single-stranded gap region in the viral genome is repaired by the viral pol protein thereby circularizing it into a covalently closed circular DNA (cccDNA) form, which serves as a template for transcription of genomic RNA.[18–21]

The *X* ORF encodes a 16.5-kd protein (HBxAg) which is involved in signal transduction, transcriptional activation, DNA repair, inhibition of protein degradation and may contribute to the oncogenic potential of HBV.[22,23]

Two direct repeats (DR1 and DR2) in the 5′ ends of the plus strand are required for strand-specific DNA synthesis during replication while the enhancer elements, En1 and En2, drive transcription of liver-specific expression of viral gene products.[9,24] The HBV genome encodes seven proteins: HBx, core, polymerase, L-, M-, and S-HBsAg, and pre-core/HBeAg[9] (Table 1).

Epidemiology and Transmission

Patients infected with the HBV can transmit it to non-immune recipients who do not have hepatitis B surface antibody (are anti-HBs negative).[43] The mode of transmission may vary in different geographical areas.[44] In high prevalence areas, mother-to-neonate transmission is the predominant mode of transmission. To clarify here, the perinatal period is defined as beginning at 28 weeks of gestation and ending at 28 days after delivery. Therefore, the term "mother-to-neonate" is more specific in indicating peri- and postpartum transmission. In low-prevalence areas, the viral pool is maintained through sexual transmission between parents and subsequent mother-to-neonate transmission. In areas with intermediate prevalence, most infants get infected through horizontal transmission during early childhood.

Mother-to-neonate Transmission

Mothers can transmit the HBV to their fetuses *in utero* in 3–9% cases.[45,46] These infants do not respond to postnatal vaccination and/or administration of immunoglobulins. Hepatitis B virus has been detected in the villous capillary endothelial cells and trophoblasts of the placenta. The risk of transmission through this route can increase in the presence of high maternal viral load and preterm labor. The exact mechanism for prenatal transmission of HBV is not clearly known, but there are several possibilities as follows: (a) Transplacental leakage of HBeAg-positive maternal blood, if there is a disruption of the placental barrier;[47] (b) placental infection with trans-placental transmission;[45] (c) through infection in oocytes or spermatozoa, which can contain HBV DNA;[48] and (d) ascending vaginal secretions from an infected mother *in utero*.[45]

More than 90% of the cases of neonatal hepatitis B infections occur during the peripartum period.[49] Passive and active immunization of neonates born to HbsAg-positive mothers within 12 hours of birth can reduce the risk of HBV transmission by >95%.

Infants born to HBeAg positive mothers may remain at the risk of infection (9% rate of infection) with HBV even if they have received complete immunoprophylaxis and therefore they must be kept under close monitoring.[50] Those born to hepatitis B-infected mothers are at increased risk of acquiring the infection if the mother is HBeAg positive and/or has high levels of HBV DNA.[51] The infection is transmitted in 9–39% of highly viremic mothers despite postnatal vaccination (maternal DNA less than 10^5–10^6 IU/mL). In infants with borderline positive results, there may be a need for comprehensive evaluation as some may show altered HBsAg antigenicity.[52]

Table 1: Major structural components of HBV

Structure	*Available information*
Lipid envelope	The nucleocapsid is surrounded by a lipoprotein envelope derived from the nuclear membrane of the infected host cell.[25]
Glycoproteins	Projecting from the lipid envelope are viral glycoprotein spikes that bind specific host receptors to facilitate virus entry. Hepatitis B virus envelope has three viral surface glycoproteins—large, medium, and small proteins (LHBs, MHBs, and SHBs). Their expression is directed by the S gene including three start codons and one stop codon in a single ORF.[11,26]
Receptor-binding motifs	These are involved in virion attachment to cell surface receptors to allow the internalization of the virus particle. The primary target organ of HBV is hepatocytes, wherein HBV-BF, IL6, heparin sulfate, and lipoprotein lipase receptors have binding motif for preS1 region of HBV.[27,28]
Envelope protein	Hepatitis B virus particles are generated by budding of preformed cytoplasmic nucleocapsids into endoplasmic reticulum (ER) membranes containing the three viral envelope proteins (L, M, and S).[29]
Membrane protein	Either not expressed or relevance unclear fetal/infantile disease.
MHC or HLA proteins	Hepatitis B virus core antigen epitopes presented by HLA-A2 induce epitope-specific $CD8^+$ T-cell response.[30] Some MHC class II alleles have been found to confer protection against persistent HBV infection.[31]
Spike protein	Projecting from the lipid envelope are viral glycoprotein spikes that bind specific host receptors to facilitate virus entry.[32]
Surface tubules	During chronic infection HBsAg is expressed in large excess as non-infectious quasi-spherical particles and tubules that are about 22-nm diameter.[33]
Palisade layer	Either not expressed or relevance unclear fetal/infantile disease.
Viral tegument	Either not expressed or relevance unclear fetal/infantile disease.
Lateral bodies	Either not expressed or relevance unclear fetal/infantile disease.
Capsid	The capsid protein (Cp) packages the viral pgRNA and polymerase to form the HBV core.[34]
Capsomeres	The proteins that form the structural unit of the capsid may form three-dimensional structures known as capsomeres that are visible in an electron micrograph. The core proteins of the capsid bind the nucleic acid through a carboxy-terminal protamine region that contains nucleic acid-binding motifs organized into four repeats.[35]
Core membrane	Core membrane plays a role in nucleocapsid assembly as proven by a decrease in core membrane association coinciding with impaired nucleocapsid formation as a result of improper sorting and trafficking of core to assembly sites in Rab33B-knockdown cells.[36]
Protein core	The core (capsid) protein of HBV is the building block of nucleocapsids and mediates virus–host cell interactions in persistent HBV infections. It has a pleiotropic role in HBV replication, thereby making it an attractive target for antiviral therapies of chronic hepatitis B.[37]
Core fibrils	Either not expressed or relevance unclear fetal/infantile disease.
Matrix	The cytosolic matrix domain (MD) located between amino acids (AA) 103 and 124 of the large HBV envelope protein L is essential for virion formation.[38]
Enzymes	Hepatitis B virus polymerase is the best known enzyme in the HBV; has roles in protein-priming, RNA- and DNA-dependent DNA synthesis, and ribonuclease H activities.[39]
RNA elements	Hepatitis B virus replicates through an RNA intermediate. The pgRNA acts as a template for RT and also as a messenger RNA for core and polymerase. The pre-core RNA is involved in the translation of the pre-core gene product.[10,24]
Nucleus	Either not expressed or relevance unclear fetal/infantile disease.
Nucleosome	Either not expressed or relevance unclear fetal/infantile disease.
DNA	Hepatitis B virus has a partially double-stranded circular DNA genome of about 3.2 kb pairs.[40]
RNA	No RNA genome exists.
Genome-associated polyprotein	Either not expressed or relevance unclear fetal/infantile disease
DNA polymerase	HBV polymerase (P) protein is a four-domain multifunctional enzyme that has protein-priming, RNA- and DNA-dependent DNA synthesis (i.e., RT), and ribonuclease H activities.[41]
RNA polymerase	All known HBV RNAs, including the subgenomic, pregenomic, and precore RNA, are transcribed by cellular RNA polymerase II using cccDNA as the template.[10]
RT	Hepatitis B viruses (hepadnaviruses) replicate their DNA genomes by RT of an RNA intermediate.[42] The HBV RT has the unique ability to initiate viral DNA synthesis using itself as a protein primer in a novel protein priming reaction.[39]
Head	Either not expressed or relevance unclear fetal/infantile disease.
Base plate	Either not expressed or relevance unclear fetal/infantile disease.
Integrase	Either not expressed or relevance unclear fetal/infantile disease.
Tail	Either not expressed or relevance unclear fetal/infantile disease.
Tail fiber	Either not expressed or relevance unclear fetal/infantile disease.
Neck	Either not expressed or relevance unclear fetal/infantile disease.

The risk of transmission after amniocentesis and other diagnostic procedures during antenatal period is low and any procedure which is indicated for genetic invasive testing should not be withheld for the fear of transmission, especially when the mother is HBeAg negative with low HBV DNA load. Use of narrow gauze needles (22G) under supervision may possibly reduce the risk of transmission.[13]

The evidence for transmission with preterm rupture of membranes is uncertain, and hence, current recommendations do not suggest altering the obstetric management. Similarly, evidence for elective caesarean section to prevent the transmission is limited and is not routinely indicated for reducing the risk of transmission.[53]

Transmission through Human Milk Feeding

In neonates who are vaccinated and treated with the HBIg at birth, the chances of transmission of hepatitis B infection through breastmilk are markedly reduced.[54]

Paternal Transmission

Most of the transmissions from neonates born to HBsAg negative mothers. Infections from HBsAg positive fathers are considered to result from close postnatal contact of unprotected neonates with the infected blood and body fluids of the fathers.[55]

Clinical Presentation

The availability of vaccination and postexposure prophylaxis with HBIg has considerably reduced the risk of perinatal HBV transmission.[49] Infected neonates rarely present with biochemical or clinical signs of disease immediately after birth. They are usually asymptomatic but may develop mild, persistent liver enzyme elevations at 2–6 months of age due to chronic antigenemia (immune–tolerant phase).[56] The immune tolerant phase can persist for years and can then progress to the immune active phase.[57] A small proportion of neonates can develop acute hepatitis by 2 months of age and may present with fulminant acute hepatitis. Few may develop chronic liver disease and are at risk of cirrhosis and/or hepatocellular carcinoma.

Diagnosis

The diagnosis of hepatitis B infection in neonates, as in adults, is based on serological assays and detection of HBsAg.[58] Hepatitis B virus DNA is not recommended for screening since it may remain persistently high for decades even after clearance of HBV infection.[59] Apart from HBsAg, the detection of HBeAg, and antibodies to these viral proteins may also be useful for diagnosing the hepatitis disease in neonates. The presence of HBsAg in the infant at 1–2 months of age is indicative of vertical mother-to-infant transmission.[49] However, HBsAg can remain transiently positive in some neonates up to 21 days following hepatitis B vaccination.[60]

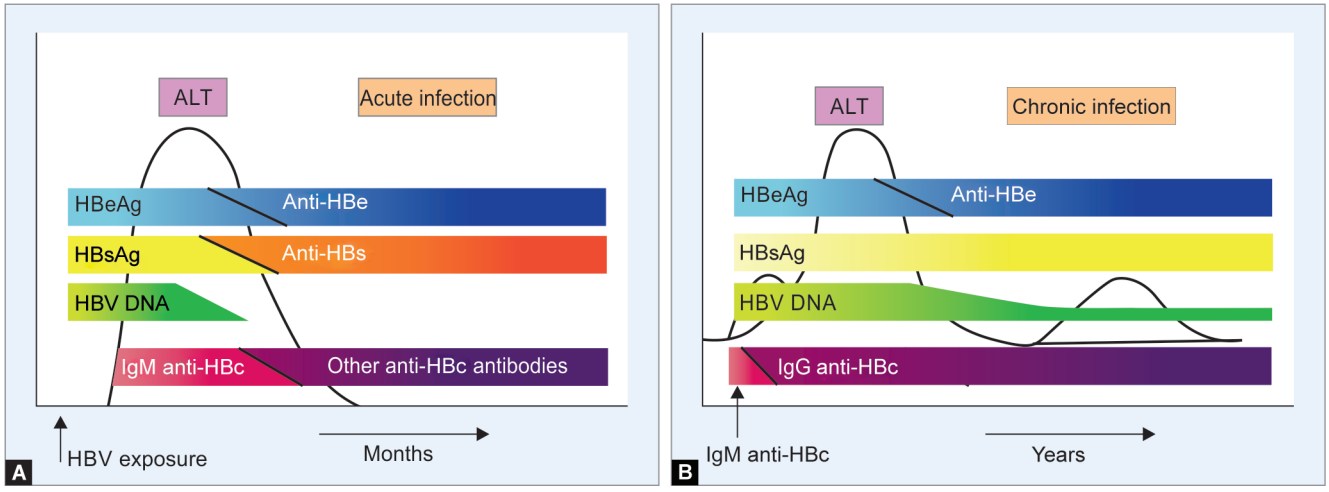
Differential Diagnosis

The differential diagnoses for elevated liver enzymes and acute liver failure presenting in neonatal period and infancy that should be kept in mind while evaluation include:[61]

- Congenital TORCHeS (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes, and syphilis) infections;
- Rarely, vertically transmitted acute infectious causes seen chronically/seasonally in certain parts of the world (malaria, dengue, and chikungunya) may present with similar clinical features;
- Neonatal sepsis causing hepatitis and liver failure;
- Inborn errors of metabolism presenting with hepatic failure (tyrosinemia, galactosemia).

Postdiagnosis Evaluation

Once a neonate or infant is diagnosed to be infected with hepatitis B, serial follow-up with monitoring of liver enzyme activity and serological status needs to be done. The association of liver enzymes and phase of Hepatitis B infection is shown in Figure 2. A few cases may also require a liver biopsy in addition to the above tests to determine the response to treatment.[62] A few studies have shown histological changes, where the liver biopsy was repeated. Histopathological changes of chronic liver disease



Figs 2A and B: Acute HBV infections are marked by a period of transaminitis lasting for a few weeks. Anti-HBc, first IgM and then other subclasses can be detected for a few weeks. This is followed by serial appearance of HBV DNA, HBsAg, and then HBeAg that last for a few weeks and are then replaced by specific antibodies lasting for a few months

and early fibrosis may indicate predisposition to cirrhosis and hepatocellular carcinoma.

In chronic infections, transaminitis can last months to years. Also, IgG antibodies against HBc become detectable soon. Hepatitis B virus DNA and HBsAg remain measurable for prolonged periods extending into years. Both HBeAg and specific antibodies can also be detected for years.

Management

The management of neonates born to mothers with hepatitis B infections includes close follow-up and monitoring of serological status.[63] Most infected neonates are asymptomatic (immune–tolerant phase) and no specific management is required.[64] The immune–active phase can be associated with acute exacerbations with elevated liver enzymes and needs to be managed per standard protocol of acute liver failure management.[65]

Some of the infants in immune active phase can have persistently elevated liver enzymes along with raised HBV DNA levels.[66] In children older than 2 years, nucleoside analogues and interferon have been used off-label, but there is a need for evaluation of efficacy in neonates.[67] The goal of medical management in these cases is to reduce the risk of transmission, to and that of cirrhosis and hepatocellular carcinoma later in life. The selection of children for treatment is primarily based on phase of chronic HBV infection and it is suggested not to treat the patients in immune–tolerant phase, who have only mild elevations of ALT (<1.5–2 times higher than normal) and high HBV DNA levels (>20,000 IU/mL).[68] Treatment in this phase can reduce that rate of seroconversion, but it has also been associated with the development of drug resistance.

Children in immune–tolerant phase, particularly those with Asian heritage may not always show a consistent response to interferon.[69] Hepatitis B virus genotype B is seen more frequently in the Asian population, which may explain the suboptimal response to these drugs.[70] However, the reasons for these unpredictable responses are not well-elucidated. The preferred oral nucleoside analogues which have been licensed for use in United States are tenofovir, disoproxil fumarate and entecavir, in children above the age of 2 years.[71]

Duration of Treatment

(a) For children and adults with persistent HBeAg, indefinite treatment may be required; (b) In adults who undergo seroconversion and become HBeAg negative, treatment may be stopped though the optimal duration has not been defined. After completion of treatment, most centers follow these infants quarterly for at least 1 year to detect any flare-ups and exacerbations.[72]

Prevention

Mother-to-neonate transmission can occur *in utero*, at birth, or later in infancy. The risk of transmission without any use of active and passive immunization in peri- and postpartum period may approach 90%, but the universal maternal HBV screening, hepatitis B vaccination of newborn infants and use of HBIG prophylactically has reduced the transmission.[1,2]

In a large cohort study from the USA, the transmission risk was noted to be high when a mother was HBeAg positive, had a high HBV viral load of above 2000 IU/mL, or had received less than 3 doses of the hepatitis B vaccine.[73] The updated CDC guidelines (2018)[1] recommend the following for prevention of Hepatitis B infection in neonates:

- All stable infants weighing above 2000 gm should receive hepatitis B vaccination within 24 hours of birth;
- HBV DNA testing for all pregnant women infected with hepatitis B infection;
- Neonates born to women with HBV infection who do not respond to the primary vaccination series and continue to show anti-HBS titers above 10 mIU/mL, should be considered for single-dose revaccination.

Hepatitis B vaccination is the mainstay of preventing these infections.[74] Hepatitis B immunoglobulin can protect exposed infants for up to 3–6 months following perinatal exposure.[1] The presence of anti-HBsAg indicates immunity against HBV infection. Infants with vaccine-induced anti-HBsAg levels >10 mIU/mL are generally considered seroprotected. The three-dose hepatitis B vaccine series usually induces a protective antibody response (anti-HBS) >10 mIU/mL) in nearly 95% of healthy infants.[75]

The birth vaccine dose acts as postexposure prophylaxis for neonates born to HBV-infected mothers.[49,74,75] Hepatitis B vaccine and HBIg can prevent 75% and 71% perinatal transmission, respectively.[76] In combination, the efficacy approaches 94%. The two single-antigen vaccines approved for use in the United States and many other countries are Recombivax HB (Merck & Co., Inc., Whitehouse Station, New Jersey) and Engerix-B (GlaxoSmithKline Biologicals, Rixensart, Belgium). However, despite all approvals, currently available evidence is of very low-to-low quality, and we do not know with certainty whether antenatal HBIG administration has an effect on the proportion of newborns with HBsAg and HBV-DNA compared with no treatment[77] (Flowchart 1).

Revaccination

The HBsAg negative infants with anti-HBsAg levels below 10 mIU/mL should receive a single dose of the hepatitis B vaccine and then tested 1–2 months later for anti-HBS antibody levels.[1] Infants who still have titers below protective levels should be considered for a second 3-dose series of hepatitis B vaccination and then the titers should be measured 1–2 months later.[1]

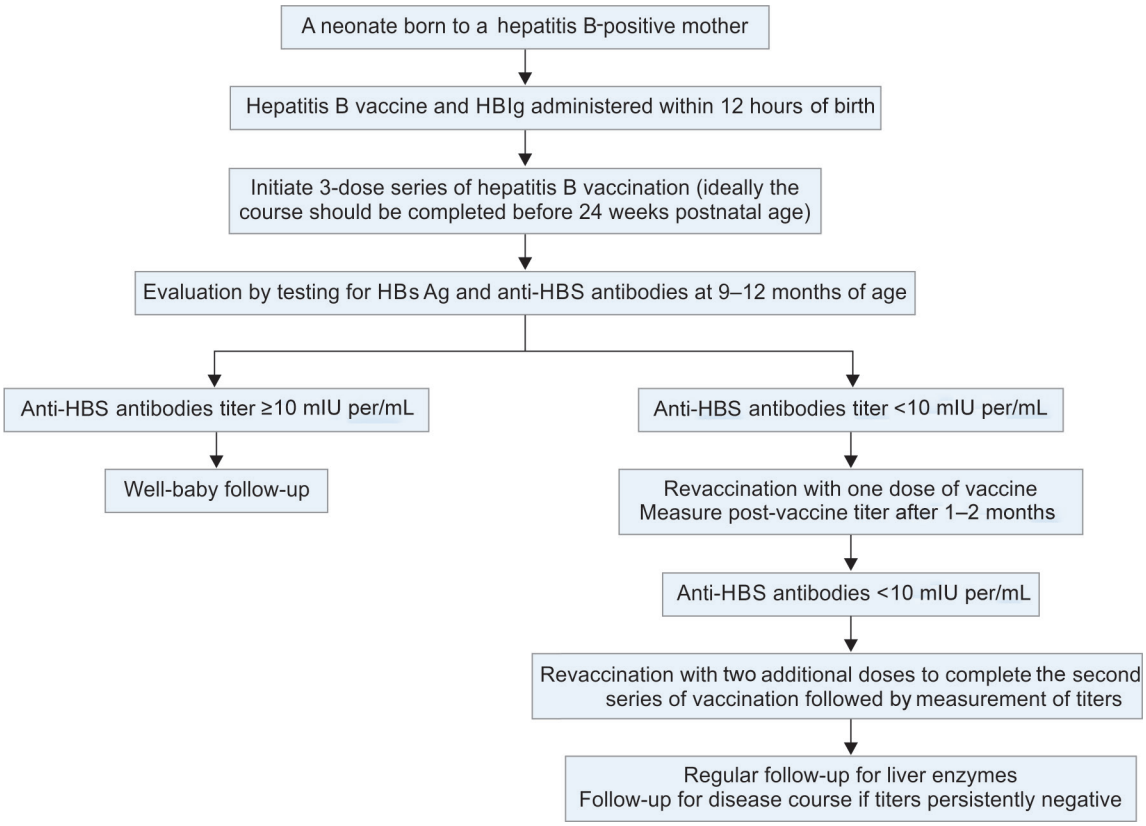
Maternal Antiviral Therapy for Preventing Perinatal HBV Transmission

All pregnant women irrespective of previous testing and vaccination status, should be tested for HBsAg during first trimester (WHO guidelines 2020).[78] Women who test positive for hepatitis B infections should be tested further for HBV DNA levels. In addition, they should be counselled about the concerns associated with hepatitis B infections and the need for antiviral prophylaxis/treatment for the neonate.

The WHO (2020) recommends that pregnant women who test positive for HBV infections with HBV DNA levels ≥ 5.3 $\log_{10}$ IU/mL (≥ 200,000 IU/mL) should receive tenofovir prophylaxis from the 28th week of pregnancy until at least the time of birth, to reduce the risk of transmission of the virus to the infant.[78,79] The systematic review commissioned by WHO on 129 studies indicated a protective effect regardless of the antiviral used for prevention (Tenofovir 300 mg: odds ratio [OR] 0.16, 95% confidence interval (CI): 0.10–0.26; lamivudine 100 mg: OR 0.17, 95% CI: 0.13–0.22; telbivudine 600 mg: OR 0.10, 95% CI: 0.08–0.13).[80]

WHO recommends that if antenatal HBV DNA testing is not available, HBeAg testing can be used as an alternative to HBV DNA testing to determine eligibility for tenofovir prophylaxis, to prevent mother-to-child transmission of HBV.[80] The risk of

Flowchart 1: Management of neonates born to HBV-infected mothers



perinatal transmission is reduced drastically with timely initiation of treatment of affected pregnant women at 28–32 weeks with administration of Hepatitis B vaccine and HBIg immediately to the neonate immediately after delivery.[49] The drugs used in antenatal period are lamivudine and tenofovir, and due to lesser reports of resistance with tenofovir use, it is being preferred for maternal management of hepatitis B infection.[81]

Long-term Outcomes and Prognosis

The natural course of hepatitis B infections depends on the interplay between the viral antigen and the immune response. Perinatally-transmitted infections typically have a phase of immune–tolerance that may last for 10–30 years.[64] The infected individual usually remains clinically asymptomatic despite having high levels of HBV DNA, and the biochemical evidence of liver dysfunction remains low. The transition from an immune–tolerant to immune–active phase can happen years later, and then be associated with acute exacerbations or manifest as chronic failure.[69]

The rate of transition from acute to chronic phase is determined predominantly by age at infection and is approximately 90% for perinatally acquired infections, 20–50% for infections occurring between the ages of 1–5 years, and less than 5% for infections acquired during adulthood.[49,82] These findings underscore the importance of birth vaccination to prevent persistent infections with long-term liver dysfunction. Progression to the chronic state and cirrhosis predisposes to other complications and nephropathy, aplastic anemia, and hepatocellular carcinoma.[61]

There have been reports of spontaneous clearance of HbsAg.[83] In most of these infants, the clearance of HBsAg indicated good prognosis. The cumulative rate of spontaneous HBeAg clearance is estimated to be approximately 2% during the first 3 years and about 15% after 20 years of infection.[84] The low rate of viral clearance in adolescence and early adulthood accounts for the high frequency of maternal-infant transmission in Asian countries.[85]

The progression rates at different stages have been estimated as below:[86]

- Chronic hepatitis to cirrhosis – 12–20%;
- Compensated cirrhosis to hepatic decompensation – 20–23%;
- Compensated cirrhosis to HCC – 6–15%.

The cumulative survival rate for compensated cirrhosis is 85% at 5 years.[87] For decompensated cirrhosis, it is between 55% and 70% at 1 year and 14–35% at 5 years.

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REVIEW ARTICLE

Establishment of the First Religiously-compliant Human Milk Bank in Bangladesh

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Received on: 26 November 2022; *Accepted on:* 27 November 2022; *Published on:* 23 December 2022

Abstract

Human milk banks (HMBs) collect, screen, process, and dispense donated human milk (HM). There are more than 500 large HMBs in the world but only a few are functioning in Muslim countries, and that too on a limited scale. Human milk banks that are similar to those in the Western countries have been difficult to establish in Muslim countries as Islamic laws do not allow the consumption of unidentified donated milk from multiple donors. Human milk is known to be important for nutrition in premature and critically ill infants, and so there is a well-recognized need to develop religiously compliant and conditionally identified HMBs in Muslim countries. In these milk banks, every mother's milk is processed and stored separately, and the milk provided by one mother can be provided to an infant from a different family only after appropriately counseling both families about the Islamic laws of prohibition of future marriages between milk siblings. Documents related to these issues are provided to both families and data need to be maintained for future reference. In this article, we recount the educational, financial, and infrastructural challenges that we faced in establishing religiously-compliant HMB in Bangladesh. There is already a noticeable reduction in infant mortality in our region.

Keywords: Breastmilk, Human milk bank, Large for gestational age, Microbiological screening, Newborns, Premature, Triglycerides.

Newborn (2022): 10.5005/jp-journals-11002-0047

Introduction

Human milk banks are an important asset in the care of premature and critically ill newborn infants who do not have access to their mother's own milk (MOM).[1–3] These services collect, screen, store, process, and dispense HM donated by nursing mothers, who may be biologically related or not, for feeding the recipient infants.[3,4] The World Health Organization (WHO) emphasizes that the best alternative when a biological mother is not able to breastfeed her infant(s) is to use HM from other sources. They have estimated that if every woman all over the world optimally breastfed her baby, the lives of 800,000 young children could be saved every.[5,6] Recently published research and systematic reviews support the conclusions that breastfeeding and HM are the reference normative standards for infant feeding and nutrition.[7] Beneficial effects of donor milk remain significant and donor milk is still highly-preferable when MOM is not available.[8]

Human milk is the best source of nutrition for all newborns, whether they are born at term or preterm; or are appropriate-, small-, or larger-for-gestational age.[3,8] It contains a variety of bioactive factors, which are known to promote the maturation of the infant's immune and digestive systems.[6,9] Human milk is known to protect against necrotizing enterocolitis (NEC) and neonatal sepsis.[7] The American Academy of Pediatrics has also recommended donor human milk (DHM), even if in part, due to a reduction in occurrence of NEC.[4,10] A systematic review and meta-analysis of data from clinical trials shows that DHM has a protective effect against NEC in preterm and low-birth weight when compared to formula.[11] Unfortunately, MOM is not available at all or in insufficient quantities in many infants for some social, biological (premature birth, maternal illness, or drug intake), or yet unclear reasons.[12]

If MOM is not available or sufficient for an infant, milk donated by other women or a wet nurse that is processed, verified for safety, and stored appropriately in a HMB may be of help.[13] In North America, HMBs have provided milk from a single donor or by pooling the milk from up to 5 women before distribution.[14]

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How to cite this article: Rahman MM, Khatun S, Kabir N, *et al*. Establishment of the First Religiously-compliant Human Milk Bank in Bangladesh. Newborn 2022;1(4):376–383.

Source of support: Nil

Conflict of interest: None

The first HMB was established in 1909 in Vienna, Austria.[15] Since then, many such facilities have been established and at least 500 large HMB organizations are currently active in 38 countries worldwide.[2] Brazil has an extensive network of 217 HMBs, and is considered to have the most cost-efficient system of milk banking in the world.[16] However, despite all progress, the HMB movement remains constrained in the Muslim world.[17–20] We have been able to establish a sizeable HMB service in Dhaka, Bangladesh, despite some resistance during its inception.[21,22] In this article, we recount the educational, financial, and infrastructural challenges that we faced in establishing this religiously compliant and conditionally identified HMB.[23] These services have already begun to positively impact the infant mortality in our region.[21]

Human Milk Bank Movement in the Islamic World

In Muslim countries, the development of HMB facilities has remained constrained in number and scale.[14,16–20,24–36] The Islamic tradition recognizes breast milk as the optimal source of nutrition for infants, but religious-ethical reservations have curtailed the enthusiasm to develop HMBs.[37] There are a few notable, pioneering HMBs in Turkey, Kuwait, Iraq, Iran, Pakistan, and Malaysia.[24,25,33,35,38] Kuwait, Iran, and Malaysia have a few more facilities in planning.[25] There is a need for religious education so that the involved parties truly understand the message from the leaders.[17] We also need to develop more religiously compliant, conditionally identified HMBs, which will be different from those in the West where milk from many mothers can be pooled and pasteurized.[35] There is a need to establish norms for developing HMBs in the Muslim world to protect the best interests of infants while respecting the tradition of milk kinship.[38,39]

One possible religiously compliant option for Islamic families is to promote HMBs is to offer milk from only one donor to a baby.[20] The identity (ID) of the donor and recipient needs to be carefully preserved, and the information should be shared with both families.[17,40] Juggling a baby and a full-time job is always a challenge for a mother anywhere in the world.[41] The problems have been particularly difficult in the promotion of HM feeding for us in Bangladesh, and so we wanted to share our experience as we are just beginning to develop the HMB movement. In Bangladesh, about 3.6 million women are employed in the garments industry, which is a particularly labor-intensive and time-consuming effort.[42] Many of these industrial facilities do not yet have dedicated space for nursing mothers to express milk in comfortable privacy.[43] To develop wholesome solutions, mothers need the means to transport/ship the expressed HM in a timely fashion, and possibly crèche facilities that would empower them to carry their growing infants to their places of work for periodic feeding. Workplaces should be in accordance with International Labor Organization Maternity Protection Convention 2000 (No. 183) Article 9:1.[44] A woman should be provided with the right to one or more daily breaks or a daily reduction of hours of work to breastfeed her child. To achieve this goal, there is a need to develop HMBs; these are the best institutionalized and safely-established repositories of donated milk.[45]

Some of this information about how working women can best comply with the religious guidance can be accessed on the web at http://www.nicuicmh.org. We will also provide smartphone apps about the HMB facilities to the donor and recipient families for their future reference. To assist them in complying with the Islamic laws, we also explain and provide the Surah An-Nisa, the Surah-4, verse-23 of Al-Quran,[46] with translations in Bangla and English to promote understanding. The following verse is particularly important:
Forbidden to you (for marriage) are: your mothers, your daughters, your sisters, your father's sisters, your mother's sisters, your brother's daughters, your sister's daughters, your foster mother who gave you suck, your foster milk suckling sisters, your wives' mothers, your step daughters under your guardianship, born of your wives to whom you have gone in – but there is no sin on you if you have not gone in them (to marry their daughters), the wives of your sons who (spring) from your own loins, and two sisters in wedlock at the same time, except for what has already passed; verily, Allah is Oft-Forgiving, Most Merciful.

Religious and Moral Guidance

We sought guidance from renowned religious scholars and clerics in Bangladesh. In Islam, two infants who have been fed milk from one woman are perceived as "milk siblings".[36,47] It is both illegal and sinful for two milk siblings to marry each another.[20,36]

We have established a process to obtain HM in a medically-appropriate/safe and religiously compliant way in our medical center. There is a sequence of sequential steps:

- We provide preliminary verbal guidance and written/printed information to both the donor and the recipient groups.
- Both groups should express awareness of the following:
  - Various medical options that are available.
  - How the Islamic laws view the acceptance of donor milk and understanding of the religious concept of milk siblings.
  - Need to consent to prevent future marriages between milk siblings.
  - Need to prevent breastfeeding-related relationships as these are illegal according to Islamic laws.
- We provide a complete information pack containing a consent form, donor and recipient ID cards, donor and recipient voter ID cards, hospital discharge papers mentioning the names of baby's own biological mother and father, milk mother's name, photographs of the donor and the recipient.

Need for Human Milk Bank in Dhaka

Our neonatal intensive care unit (NICU) at the Institute of Child and Mother Health, (ICMH), Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh is an established level III care center, where we have now treated several thousand critically ill patients every year for several decades.[48] We treat both inborn and outborn babies, and a large proportion of these infants can benefit from access to HM. However, as in every NICU, not every infant has access to MOM due to maternal illness, ongoing drug treatment, or even the death of their mothers during labor or due to concomitant illness(es) that might become more severe during pregnancy.[49,50] We also have a second set of families where the mother is able to produce more milk than her infant needs, and she has to discard her precious milk to find comfort from breast engorgement and/or mastitis.[51] Our team has long wondered about whether we could utilize this HM while remaining compliant and maintaining our reverence for the Islamic law.

Development of a Religiously Compliant Human Milk Bank in Bangladesh

The idea of establishing an exceptional HMB in Bangladesh was conceived in January 2019 following visits to HMBs in Mumbai, India and in Valencia, Spain.[52,53] After reviewing the Islamic laws and scientific literature, we formally requested the Director General of Health Services (DGHS)[54] and the Ministry of Health and Family Welfare[55] for approval of an HMB. We also applied to the Islamic foundation[56] for religious clarification and to get a Halal certificate.[17,20,57] Several meetings were held with the administrators and the Islamic group. The facilities were equipped in the first week of November 2019 and the staff members were trained in the subsequent 3 weeks. The HMB was inaugurated in December 2019.[58]

Infrastructure

The HMB facility has well illuminated rooms with at least 500 square feet carpeted area, which are situated near or within the NICU or in the facility for kangaroo mothering care corner.[48] These areas are designed to provide optimal hygiene, comfort, and privacy. It has several compartments including the following:

- A waiting area with reception and crèche.
- A counseling area.
- An area for donation/collection of HM that is designed to help maintain the privacy of the donor.
- Working space for personnel and storage of equipment.
- A toilet block within the facility.

Equipment

The milk bank facility is equipped[59,60] with the following (Fig. 1):

- A pasteurizer with special shell to pasteurize small amount Individual mother's milk (Fig. 1A).
- A laminar airflow machine (Fig. 1B).
- A hot air oven (Fig. 1C).
- Steeliness steel containers labeled with heat- and water-resistant ID numbers (Fig. 1D).
- Deep freezers (Fig. 1E).
- High-quality breast pumps (Fig. 1F).
- A data–entry system and ID card printer.

We currently collect, process, and preserve 50–250 mL of HM daily, which can be served to 50–100 newborns.

Compliance with the Islamic Laws

Our HMB is running according to the Islamic laws.[19,20,36] Critically ill newborns whose lives are not sustainable without breast milk are prioritized for service. Islamic literature is provided to both the donor and the recipient groups.

- Activities of this HMB have been supervised by the Ministry of Health and Welfare, DGHS and the Islamic Foundation.[54–56]
- If the infant's parents follow a different religion, their laws of that specific religion are followed.
- No financial exchanges are done between donor and recipient groups.
- Milk of one mother is given only to one infant. Donor and recipient receive ID cards with specific information, including

Figs 1A to F: Equipment used in our HMB. (A) Pasteurizer. We use small-volume shells to pasteurize aliquots of milk as small as 25 mL; (B) Laminar airflow to combat external contamination; (C) Hot-air oven to sterilize milk containers at 300°C; (D) Stainless steel container with a heat- and water-resistant ID sticker; (E) Deep freezer that can store milk at –26°C for up to 18 months; and (F) High-quality breast pump that can help express 250 mL or more milk within 15 minutes. The personnel staffing these areas include trained physicians, nurses, one computer operator and one counselor to run this bank properly.

the address of both groups, their telephone numbers, and email addresses. The milk bank is run under the supervision of a committee that includes a Mufti, a journalist, nutritionist, pathologist, public representative, and the leadership of the milk bank, Department of Pediatrics, and the ICMH.

- Milk is collected from mother after proper medical checkup, screening and consent. Individual mother's milk is collected, processed by pasteurization and preserved separately in separate food grade container after labeling with a sticker. Consent papers, which include photographs of the donor and recipient are given to both groups.
- Every year, an annual report is provided to the district marriage registration.
- All information is preserved online at the website www.nicuicmh.org and in the smartphone apps of the HMB for future reference.
- A compact disk with video recordings of the information is given to both groups.
- Milk mother's and father's name is mentioned in the hospital discharge papers along with own father's and mother's name.
- In near future, we plan to include the milk mother's and father's name in the national ID cards as needed.

Collection of Human Milk

After proper counseling, checking suitability for donation, getting written informed consent, obtaining detailed medical history, physical examination, and laboratory tests, the donor is sent to a designated HM collection area in the HMB. Milk is collected by trained staff with hygienic precautions by manual expression, which has the advantages of low cost, high efficacy, and low risk of contamination.[61] Donors who want to donate regularly can also simultaneous bilateral breast expression by using good quality breast pumps.[62] Milk is collected in properly labeled sterile container and transported to HMB under cold storage condition.[63] All data of donor and recipient with contact number is to be maintained in soft- and hard-copy records.

Processing of HM

All batches of collected raw breast milk is refrigerated immediately until the serological reports are available and reassuring.[64] Fresh raw milk is not added to the frozen milk since this can result in deep freezing with hydrolysis of triglycerides.[65] When fresh raw breast milk to frozen raw breast milk previously collected from same donor, it is chilled before adding to frozen milk.[59] For sick or preterm babies, it is advisable to use a new container for each pumping; we are constantly evaluating various approaches for optimal storage.[66]

Individual mother's milk is pasteurized in individual containers and no pooling and mixing is carried out from multiple donors.[18,36] Pasteurization is carried out by Holder method.[67] If the cost issues are manageable, microbiological screening of donor milk is done as soon as possible after pasteurization.[68] No bacterial growth is acceptable in post-pasteurization microbiology cultures.[69]

Storage of Processed HM

Prior to the availability of culture reports, pasteurized milk is kept in dedicated freezers and not disbursed.[70] It is stored in the same container that is used for pasteurization; it is advisable not to transfer processed milk in other containers as it has risk of contamination.[71] Culture negative processed milk should be kept at –26°C in tightly-sealed containers that are labeled with clearly mentioned expiry dates and other relevant data. It can be preserved for 3–18 months.[72] Random cultures of preserved milk before disbursal can aid quality assurance.[73]

Disbursal

Processed milk is disbursed at the requisition from the physician after informed consent from the parents of the recipient. Preterm babies are prioritized to milk from preterm donors on a first-in-first-out basis from the storage. Transport of milk should be done under cold storage in the same pasteurized container until it is used. Frozen milk is usually thawed by either defrosting the milk rapidly in a water bath at a temperature not exceeding 37°C, or under running lukewarm water.[74] We ensure that the cap of the container does not come in contact with the water as it is likely to get contaminated.

Human milk should never be thawed in a microwave as this can reduce the immunoglobulin A (IgA) content.[75] Also, HM should not be refrozen after being thawed as this increases the hydrolysis of the triglycerides in the milk.[65] While bringing to room temperature, it should be gently agitated to make a homogenous mixture before use.[76]

Labeling and Record-keeping

Human milk bank should have an operational objective of ensuring full traceability from individual donation to recipient, and maintaining a record of all storage and processing conditions. Written standard operating procedures should be followed. Confidentiality of records should be maintained by the milk bank. Proper labeling at all levels is mandatory.[25] Labels should be water resistant and names and identifying details of donors, dates of pasteurization, batch numbers and expiry date should be clearly readable.

General Guidelines for Staff of the Human Milk Bank

Standard operating procedures of the milk bank[77] should be displayed at proper places:

- Hygienic practices such as proper handwashing, donning gowns, mask, gloves, trimming nails, and locking long hairs should be maintained.
- Gloves should be worn and changed between handling raw and heat-treated milk;
- Staff should undergo regular health checks and be immunized against hepatitis B.
- There should be a program for ongoing training of the staff.

Importance of Human Milk Bank in COVID-19 Pandemic

Standard recommendations during the COVID-19 epidemic suggest that who test positive mothers can feed their babies with infection control and prevention measure.[78–83] If the mother-baby dyad test positive for COVID-19, breast feeding is not contraindicated.[84] If both the mother and her newborn are hospitalized and separated from each other, expressed breast milk can be given.[79] If there is an HMB, expressed HM can be stored and will be able to provide milk to her own baby.[85]

Economic Implications

The cost-effectiveness of using banked HM in the NICUs is established in Western countries, largely because of lower rates of NEC.[10,86–89] The costs of running a HMB in Bangladesh have not

Figs 2A to C: Changing public perception of HMBs in Bangladesh. (A) A critically ill infant with multi-system organ failure; (B) With improving survival of critically ill infants such as in image in subpart (A), we are seeing increasing public interest in ways to salvage these infants. Human milk is finally getting its well-deserved recognition as a therapeutic measure. The panel shows a newspaper article and the photograph on the right is from our NICU that was published in social media; the images show changing public perception of HM as a treatment measure. With their financial constraints and low hopes of survival of the infants, many of these infants used to be abandoned by their families; (C) Progressive improvement in infant mortality rates have now encouraged parents to seek previously unrecognized therapeutic measures such as use of banked HM. All images reproduced after due parental consent.

been specifically evaluated yet. However, as prematurity and sepsis are two major causes of neonatal mortality in Bangladesh similar to other countries,[90] early initiation and ensuring exclusive HM feeding can reduce the burden of sepsis and reduction of neonatal mortality by nearly a fifth.[91] Human milk feedings can be a major cost savings for the nation.[92] (Figs 2A to C).

Conclusion

In the Western countries, establishing HMBs is a common initiative for the benefit of premature and critically ill newborn infants who are unable to receive their mothers' milk for various reasons. However, in Muslim countries, the development of such facilities remains constrained in capacity, location, and the number of potential beneficiaries. The Islamic tradition recognizes breast milk as the optimal source of nutrition for infants, but there are religious-ethical reservations in developing public facilities to share HM. We have successfully developed a religiously compliant and conditionally identified HMB in Bangladesh that strictly follows the guidelines of the Islamic laws. There is a need for careful collection and preservation of the identifying information of our patients and their families to prevent any marriages between milk siblings. We have been able to convince our social, administrative and the learned religious leaders about the need and potential benefits of this service. This model system can be replicated in other Muslim nations as it has the potential to save the lives of critically ill infants.

Acknowledgments

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REVIEW ARTICLE

Fats in Human Milk: 2022 Updates on Chemical Composition

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Received on: 15 November 2022; *Accepted on:* 25 November 2022; *Published on:* 23 December 2022

Abstract

Human milk (HM) feedings are important for all newborn infants. Healthy term infants grow well with the mother's own milk (MOM), be it in direct breastfeeding or when fed expressed breastmilk. Premature and ill infants being treated/monitored in neonatal intensive care units (NICUs) also recover better when fed with HM diets, which can include MOM, donor milk (DM), or a combination of both. In terms of chemical composition, it contains 3–5% fat, 0.8–0.9% protein, 6.9–7.2% carbohydrates (calculated as lactose), and 0.2% mineral constituents. In this review, we present the latest information on HM fats, including triglycerides, phospholipids, triglycerides, cholesterol, glycoproteins, and enzymes. This article is intended to initiate a series of periodic updates on the scientific information available on HM fats. It contains some of our own research findings with an extensive review of the literature. To avoid bias in the identification of studies, keywords were short-listed *a priori* from anecdotal experience and from PubMed's Medical Subject Heading (MeSH) thesaurus. We then searched the databases PubMed, EMBASE, and Science Direct.

Keywords: Donor milk, Infants, Mother's own milk, Neonate, Neonatal intensive care unit, Newborn, Premature, Triglycerides.

Newborn (2022): 10.5005/jp-journals-11002-0050

Key Points

- Human milk feedings are important for all newborn infants. Healthy term infants grow well with MOM, be it in direct breastfeeding or when fed expressed breastmilk. Premature and ill infants being treated/monitored in NICUs recover better when fed with HM diets, which can include MOM, DM, or a combination of both.
- Fats in HM are an important source of energy for enterally fed growing term and premature infants. Human milk fats, including triglycerides, phospholipids, triglycerides, cholesterol, glycoproteins, and enzymes may facilitate recovery in ill neonates.
- Fats account for nearly 3–5% of HM and add up to nearly 25 grams/day during the first few months after birth. The amounts are sufficient to cover the physiological needs of newborn infants of about 4.8–6.6 grams/kg per day.
- Increasing information on various types of milk fats, including triacylglycerols, phospholipids, and saturated and unsaturated fatty acids, has improved our understanding of the roles of these lipids in development and disease.
- Docosahexaenoic acid (DHA, C22:6n-3) is a particularly important polyunsaturated FA. It is highly enriched in the brain and is being recognized for its role in neurodevelopment and retinal maturation.

Introduction

Human milk feedings are important for all newborn infants.[1–3] Healthy term infants grow well with MOM, be it in direct breastfeeding or when fed expressed breastmilk.[4] Premature and ill infants being treated/monitored in NICUs also recover better when fed with HM diets, which can include MOM, DM, or a combination of both.[5] For these infants, milk may need appropriate fortification with bovine or HM-derived fortifiers.[6] The use of donor HM, not formula, is also preferred to treat term infants with transient conditions such as hypoglycemia.[7] The Joint Commission PC-05 and Baby-Friendly Hospital Initiative (BFHI) metrics include the exclusive use of HM throughout the initial newborn hospitalization.[8,9] Human milk-derived fortifiers engineered from donated HM are also becoming popular in US NICUs, and are now used exclusively in 1/3rd or more of level-3–4 NICUs.[10] The combination of HM-derived fortifiers with either DM or MOM, excluding any products cow's milk-containing products, has been labeled as exclusive HM diet.[11]

Fat in HM is an important energy source for enterally fed growing term and premature infants.[12] Infants may have developmental limitations in utilizing milk-borne fats in the first few weeks after birth.[13] The expression of many digestive enzymes and the transport of absorbed fats may be low.[14] Maternal milk also shows changes in its fat composition during this period; the carbon chain length and the degree of unsaturation show maturational changes and are important determinants of absorption.[15] The concentrations of major nutritional, immunological, and hormonal components in HM change over the course of lactation and differ between preterm and term populations.[15] These components also vary based on the infant's birth order, gender, gestational age, and postnatal chronological age.[16] Immunological factors are frequently influenced by infant's illness.[16] Some HM hormones show diurnal variations in concentration.[17] The long-term benefits of HM are promising for infants; there is a gut–lung and a gut–brain axis that

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How to cite this article: Maheshwari A. Fats in Human Milk: 2022 Updates on Chemical Composition. Newborn 2022;1(4):384–396.

Source of support: Nil

Conflict of interest: None

links the intestinal microenvironment in early infancy with gut/lung maturation and neurodevelopmental outcomes.[18,19]

In this review, we present the latest information on HM fats, including triglycerides, phospholipids, triglycerides, cholesterol, glycoproteins, and enzymes. All medical professionals involved in newborn care have long sought a comprehensive source of information on this very important topic, and after serial discussions in a subcommittee at the Global Newborn Society (GNS), we decided to collaborate across the organization to develop articles to cover various aspects of HM feedings ranging from clinical practice to its biochemistry. This article is intended to initiate a series of annual updates on the scientific information on HM. We searched extensively in the databases PubMed, EMBASE, and Scopus after short-listing keywords focused on the biochemistry and clinical relevance of these lipids.

Fats in HM

Fats (lipids) account for nearly 3–5% of HM, and are sufficient to cover the physiological needs of newborn infants of about 4.8–6.6 grams/kg per day.[20,21] About 98% are triacylglycerols (TAGs) that carry nearly 88% of fatty acids (FAs).[1,12] Small amounts of cholesterol esters (CEs) and phospholipids (PLs) are also seen.[22] Table 1 summarizes these data. Overall, TAGs, FAs, CEs, and PLs comprise the four most important classes of lipids in HM and provide 50–60% of the total HM energy content.[23] Milk fats are important vehicles for the transport of many lipid-soluble hormones and vitamins A, D, E, and K.[12] Many of these components play important roles in the development of cognitive function and visual acuity.[24]

Fats make HM energy dense without an inappropriate increase in osmotic load.[4] The highest concentrations are seen in colostrum and then decrease gradually in the postnatal period. This inverse correlation with rising milk volumes keeps the total daily ingested amounts of fats at a consistent level. The fat content shows considerable inter- and intra-individual variation.[25]

Table 1: Types of fats in HM

Type of fats	*Approximate percentages as constituents of the total fat*
Triacylglycerols	98%
Diacylglycerols	Up to 1%
Monoacylglycerols	Traces
Nonesterified FAs	Up to 0.5%
Phospholipids	Up to 1%
Cholesterol	Up to 0.5%

The percentages are rounded-off figures from our own data

There is a rise in fat content each time a mother feeds her baby; hindmilk may contain up to 6% fats, which is more than the 2% seen in foremilk.[26] There is also a diurnal variation with higher fat contents during the day and lower during the night.[17] All these variations contrast with the relatively constant concentrations of protein and lactose.

Triacylglycerols in HM

Triacylglycerols consist of a glycerol molecule that can be esterified in 3 FAs occupying segments, the sn (stereospecific numbering)-1, sn-2 (central position), and sn-3 (Flowchart 1).[20,27] In this notation, the letter C and the following numerical modifier indicates the number of carbon atoms in the FA, and the numeral after the colon is the number of double bonds in the carbon chain.[28]

Human milk contains at least 170 different types of TAGs, of which the 30 most abundant ones comprise 70% of the total milk-borne fats.[29,30] In HM, 60–86% of the TAGs are esterified and these have a unique molecular structure compared to those seen in plasma and other tissues.[31]

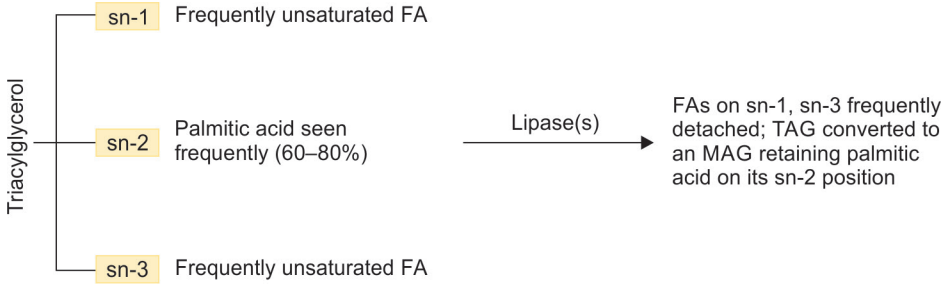
Palmitic acid (hexadecanoic, C16:0) is the most frequently seen saturated FA in HM. It is usually located on the sn-2 position and accounts for 20–25% of the total FA content.[32–34] This location is more conducive for absorption of lipids and calcium, bone health, intestinal flora, and overall comfort.[35–37] Oleic acid (18:1 ω-9) is the most frequently seen unsaturated FA in HM.[33] Fatty acids are described by the ω- or the Δ-nomenclature; the ω names show the carbons counted from the methyl end, whereas the Δ nomenclature shows enumeration from the carboxylic acid.[38] Docosahexaenoic acid (cervonic; C22:3 ω-3) is also seen frequently, located on upto 50% of the sn-2 and 42% of the sn-3 positions.[39–41] During digestion, pancreatic and gastric lipases may selectively hydrolyze FAs at the sn-1 and sn-3 positions, producing two free FAs and a 2-monoacylglycerol containing palmitic acid.[42]

Monoacylglycerols containing palmitic acid are absorbed more efficiently, even more than free palmitic acid.[35] This is because palmitic acid has a melting point (61–65°C) that is higher than the body temperature, and because it forms insoluble soaps with calcium and magnesium in the intestine.[43,44] In contrast, SFAs and 18-carbon FFAs such as oleic and linoleic acid are well-absorbed.[45] Details of various FAs seen in TAGs in HM are provided in the following sections:

Synthesis of TAGs Seen in HM

Mammary epithelial cells (MECs) contain large amounts of TAGs, most of which are synthesized locally.[46] Mammary epithelial cells contain the largest pool of FAs, nearly 95%, in the body[47]; these are acquired via two processes:

Flowchart 1: Schematic showing the structure of triacylglycerols detectable in HM



FA, fatty acid; MAG, monoacylglycerol; sn, stereospecific numbering; TAG, triacylglycerol

- Fatty acids synthesized within MECs from glucose in the endoplasmic reticulum (ER).[48] The rate-limiting step is the conversion of acetyl-CoA to malonyl-CoA, and the FA chains formed in this process grow via stepwise addition of 2-carbon units.[48] Fatty acid synthase is a key enzyme in this process.[49] Unlike in most other tissues where FA synthesis is terminated after a 16-carbon chain has been built, MECs contain an acyl thioester-hydrolase (thioesterase II) that can terminate FA synthesis at shorter lengths of 8–14 carbons to express medium- and intermediate-chain FAs.[50] These evolutionary adaptations can explain the high total FA content in milk.[51]

  Alterations in maternal diet can change the FA composition of HM.[52] Maternal diets low in fat and high in carbohydrates lead to *de novo* synthesis of FAs within the mammary gland, resulting in high concentrations of FAs of less than 16 carbons.[48] Therefore, although the total amount of fat present in the milk remains in the normal range, the fat is more saturated.

- Long-chain FAs (LCFAs; details in Table 1) imported from plasma, which are released from the digestion of TAGs (Flowchart 1) and are carried in circulating chylomicrons or very low-density lipoproteins (VLDLs).[53] These LCFAs in the bloodstream are bound to FA-binding proteins, or alternatively, combine first with CoA and then with an acyl-CoA-binding protein.[54,55]

FAs formed in these two processes bind glycerol-3-phosphate to form TAGs, and these get incorporated into microlipid droplets.[56] These droplets coalesce and move outward toward the cell membrane, where those are pinched-off into the circulation.[56,57]

Several technologies are now available to produce specifically tailored TAGs.[58] Biomimetic infant formulas containing TAG structures similar to those in HM may promote positive outcomes such as metabolic programming.[59] Lipid structures similar to those seen in HM can be produced *in vitro* by using specific lipases for interesterification.[60,61] Several types of oils and fats, such as tripalmitin, lard fat, bovine milk fat, soybean oil, canola oil, borage oil, sunflower oil, and safflower oil, have been used to produce lipid structures resembling HM fat.[62,63]

Digestion of HM-borne Saturated Fats

Neonates are developmentally deficient in many aspects of fat digestion because of low expression of lingual lipase, bile acids, and pancreatic lipase.[64] These enzymes may take a few days or more to be expressed at mature levels. Intragastric lipolysis by lingual and gastric lipases can partially compensate for the deficiency of pancreatic lipase.[64] Lingual lipase, secreted by the serous glands of the tongue, is detectable by 25 weeks' gestation.[65] Gastric lipase is secreted from the *chief (zymogen) cells* in the fundic gastric mucosa.[66] These can penetrate the milk lipid globules and hydrolyze the TAGs inside the core.[67] Fatty acids and monoglycerides resulting from intragastric lipolysis can compensate for low levels of bile acids by emulsifying lipid mixtures.[65]

Human milk feedings can cover for many deficiencies in neonatal digestion as it carries many lipases, including lipoprotein lipase, bile salt esterase, and other nonactivated lipases.[8,68,69] The composition of dietary fat should also be noted; the length of the carbon chain and the degree of unsaturation are important determinants of absorption.[70] Human milk supplies 8–12% of fat as medium-chain triglycerides (MCTs, chain length of 6–12 carbon atoms), which are hydrolyzed easier than long-chain triglycerides. In some cases, MCTs provide upto 40% of the total fat intake.[71]

Table 2: Phospholipids in human milk

	Mean (min–max) mg/100 g
Total phospholipids	25 (10–40)
Phosphatidylinositol	1 (1–2.5)
Phosphatidylserine	1.5 (1–2)
Phosphatidylethanolamine	7 (2–12)
Phosphatidylcholine	6 (2–10)
Sphingomyelin	9 (3–15)

Lipid digestion and absorption are also affected by dietary fat composition.[72] Fatty acids with shorter chain length and higher degrees of unsaturation are absorbed more efficiently without the need for lipase or bile salts.[73] Therefore, commercial formulas are often designed to contain fats with more MCTs.[71] These MCTs get digested and the FAs are transported directly to the liver via the portal vein. These MCTs can also enter mitochondria and be oxidized without the need for carnitine-mediated transport through mitochondrial membranes, and might even play a role in mitochondrial kinetics.[74,75] However, further research is needed to understand the determinants of fat absorption or improved growth in preterm infants.

Phospholipids in HM

Human milk contains traces (<1%) of phospholipids, which interact with and form a protective membrane-like covering around bioactive components such as long-chain polyunsaturated FAs and choline.[76] We have provided some of our own data in Table 2 below.

Nonesterified Fatty Acids in HM

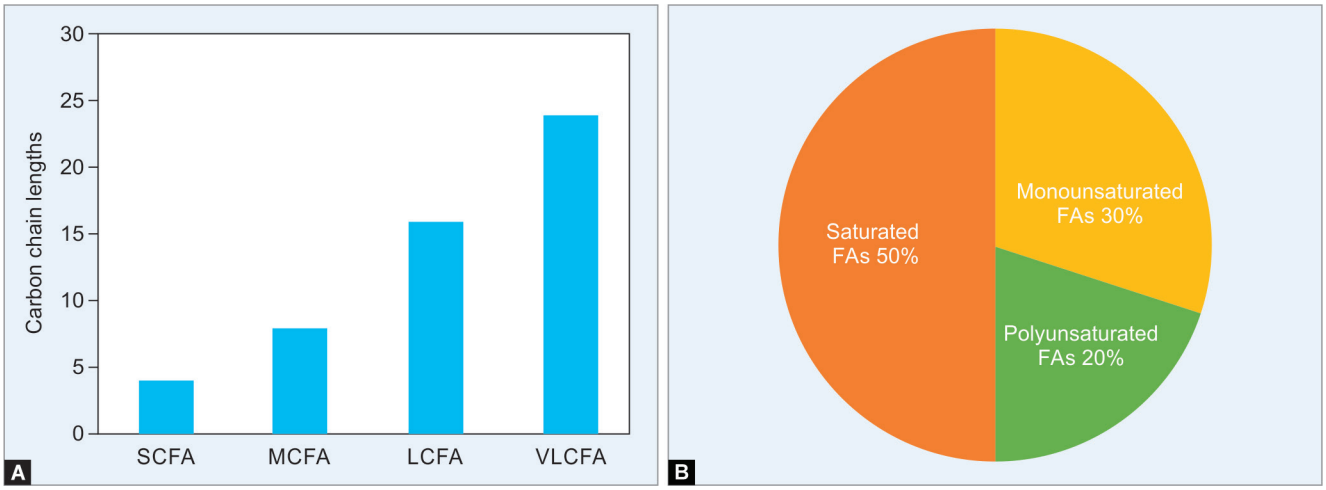
Fatty acids represent about 85% of the TAGs.[31] As shown in Figure 1A, FAs are usually classified either by (a) the number of carbon atoms, into short-chain FAs (3–5 carbons in the longest chain), medium-chain (6–12 carbons), long-chain (13–22 carbons), and very-long-chain FAs (23–27 carbons);[77] or (b) the degree of saturation into saturated, monounsaturated, and polyunsaturated categories (Fig. 1).[78] Table 3 enlists various FAs in the three categories shown in Figure 1B.

Saturated FAs in HM

About 45–50% of all FAs in HM are saturated, and these provide ≥10–15% of the dietary energy content.[79] There are small amounts of short-chain (3–5 carbons long) saturated FAs and 8–10% are medium-chain (6–12 carbons long) FAs. Triacylglycerols containing these FAs are hydrolyzed by gut lipases without the need for bile salts, and the digested products are efficiently absorbed.[80]

Besides known roles in nutrition, many saturated medium-chain FAs also perform important developmental functions.[81,82] Caprylic (octanoic acid, C8:0) and capric (decanoic or decylic acid, C10:0) promote fat absorption.[83] Capric acid also exhibits bactericidal effects.[84] The LCFAs, including myristic (tetradecanoic, C14:0), palmitic (hexadecanoic, C16:0), arachidic (icosanoic acid, C20:0), and behenic (C22:0) acids, have immunomodulatory effects.[73] Palmitic acid may alter protein acylation.[85] It is also a key component of pulmonary surfactant.[86] In HM, palmitic acid is seen in more than half of all TAGs. Oleic acid is seen in 10–12%.[1]

In HM, saturated FAs of carbon chain lengths 12, 14, 16, and 18 are seen in the highest concentrations.[1] The longer ones are a critical source of energy supply, and show dynamic plasticity.[87] Circulating stearic acid (octadecanoic, C18:0) is frequently interconverted



Figs 1A and B: (A) Structural classification of FAs based on lengths of the carbon chain (SCFA, short-chain FA; MCFA, medium-chain FA; LCFA, long-chain FA; VLCFA, very long-chain FA); (B) Classification of FAs based on saturation of carbon bonds in the fatty acid. The percentages show rounded-off data from our own laboratory

Table 3: Most frequently seen FAs in HM

Saturated FAs		*Mono-unsaturated FAs*		*Polyunsaturated FAs*	
C8:0		C14:1 ω-5		C14:2 ω-6	
C10:0	Also called capric, decanoic, or decylic acid	C16:1 ω-9		C16:2 ω-6	
C12:0	Also called lauric, or dodecanoic acid	C16:1 ω-7	Also called palmitoleic acid	C18:2 ω-6	Also called linoleic acid
C13:0		C16:1 ω-5		cis, trans-C18:2 ω-6	
C14:0	Also called myristic, or tetradecanoic acid	C16:1 ω-3		CLA c9, t11	
C15:0		C17:1 ω-7		CLA c10, t11	
C16:0	Also called palmitic acid	C18:1 ω-9	Also called oleic acid	C18:3 ω-3	Also called α-linolenic acid
C17:0		C18:1 ω-7	A C18:1 (n-7) isomer of oleic acid. Also called cis-vaccenic or cis-11-octadecenoic acid.	C20:2 ω-6	
C18:0	Also called stearic acid	C18:1 ω-5		C20:3 ω-6	
C20:0		C20:1 ω-11		C20:4 ω-6	Also called arachidonic acid
C22:0		C20:1 ω-9		C22:2 ω-6	
C24:0		C22:1 ω-9		C22:4 ω-6	
				C22:5 ω-6	

Deep shading in the box indicates levels >10%, light shading indicates 1–10%, and no shading indicates <1%

with its monounsaturated counterpart, oleic acid.[88] Such transformations can be beneficial for preterm infants who may have limitations in absorbing fats. Although there is no demonstrated benefit for energy balance or growth in growing preterm infants, there is some evidence that many saturated medium-chain FAs can be beneficial in fat malabsorption due to short-bowel syndrome and severe cholestatic liver disease.[70]

In humans, palmitic acid attached on the sn-2 position[35] renders it resistant to human lipases that can target sn-1,3 positions but not sn-2.[89] Consequently, lipase action leaves the TAGs only partially digested as sn-2 palmitate monoacylglycerols,[37,90] which have these monoacylglycerols in digested HM, are better tolerated than free palmitic acid, augment fat and mineral absorption, and may also improve bone density in the medium term.[31,37]

Litmanovitz et al.[91] used ultrasound bone sonometry for longitudinal assessment of bone density in term and preterm infants. They performed a double-blinded, randomized controlled study of bone parameters in 3-month-old term infants fed formula containing triglycerides with *sn*-2 16:0 or standard vegetable oil blends and compared those to a nonrandomized group of breast-fed infants. Infants in the intervention group had significantly higher bone density. These data were consistent with a previous report of Kennedy et al.,[92] who had used dual-energy X-ray absorptiometry and shown higher bone mass in 3-month-old infants who were fed

either formula containing structured triglycerides enriched in *sn*-2 16:0 or a conventional formula.

Palmitic acid in HM is important for lipoprotein and nonesterified FA metabolism when compared to that in TAGs sourced from plant oils.[31] In the first few days after birth, HM contains lauric (dodecanoic, C12) and myristic (tetradecanoic acid, C14) saturated FAs in low concentrations.[93] As the concentrations of these two FAs rise, that of the longer FAs decreases.[94] Early FAs may also be derived from extramammary sources, but the breast then quickly begins to synthesize these.[95] The total fat content in milk may have a predictive value; 90% of the women whose milk contained ≥20 grams fat per feeding on postnatal day 7 were successfully breastfeeding 3 months later.[96] Women with lower milk fat content had lower rates of success.[97]

Mono- and Polyunsaturated Fatty Acids in HM

Monounsaturated FAs are an important component of HM and infant formulae.[98,99] Oleic acid (OA, 18C:1 ω-9) is by far the most abundant monounsaturated FA.[99] In HM from Mediterranean regions, where consumption of olive oil is high, the concentrations of oleic acid and its congeners may exceed 40%, and the total content of monounsaturated FAs may exceed 45% of total FA in HM.[100,101] These esterified FAs in TAGs interact extensively with the polyunsaturated FAs (PUFAs).[102] We have described the interactions in the following sections:

The most frequently seen PUFAs in HM are listed in Table 1. These are derived from maternal diet, *de novo* synthesis in the mammary glands, and by mobilization from fat stores.[36] The FA composition of HM is influenced by many factors, including maternal diet, duration of pregnancy, maternal parity, and the stage of lactation.[103] Typically, the most abundant FAs are oleic (30%), palmitic (18%), linoleic (12%), myristic (6%), and stearic acids (8%).[1,104,105] Interestingly, the concentrations of palmitoleic acid (an ω-7 monounsaturated FA, 16:1 ω-7) appear to mirror those in myristic acid.93 In preterm infants, HM may not always be adequate as a source of nutrients.8

Long-chain polyunsaturated FAs play an important role in the development of the infant's brain during the last trimester of pregnancy and during the first months after birth.[106] The precursor C18 fatty acids for the n-6 and n-3 LC-PUFAs are linoleic (C18:2 ω-6) and α-linolenic acid (C18:3 ω-3).[107] These are further elongated and desaturated to form other FAs, of which arachidonic acid (AA) and DHA are essential for normal growth and development.[108] Although the LC-PUFAs are synthesized from precursor FAs in both preterm and term infants, the capacity to produce DHA and AA is not known.[109] In the first week after birth, the levels of DHA and AA might drop due to the lack of adipose reserves and insufficient FA intake by the mother.[110] The LC-PUFA content in HM in the United States, Europe, and Africa is similar, except for higher amounts of ω-3 LC-PUFAs in the milk of women whose diets contain a large quantity of fish.[111,112] Arachidonic acid (C20:4 ω-6) is an important LC-PUFA in HM.[113] Eicosapentaenoic acid (EPA, C20:5 ω-3) is seen in relatively smaller quantities.[113,114]

Polyunsaturated Essential FAs in HM

Human milk usually contains PUFAs such as linoleic acid (LA, 18:2 ω-6) and in adequate amounts.[115] These FAs promote brain growth and retinal maturation, and influence metabolism such as by reducing plasma cholesterol.[116] Human milk content of LA and

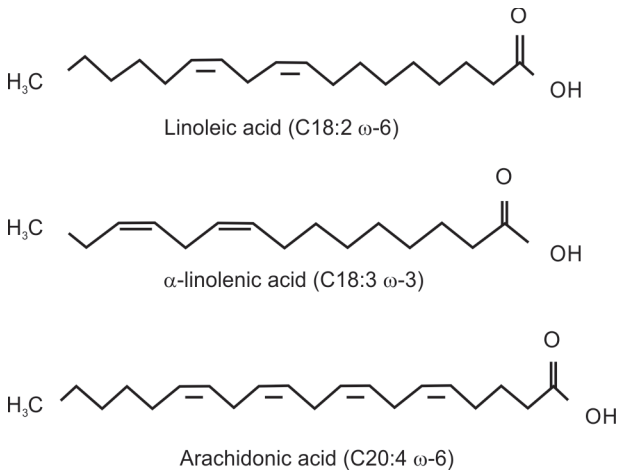


Fig. 2: The three essential FAs

alpha-linoleic acid (ALA) is known to vary according to maternal intake of these FAs.[36] The European Food Safety Authority (EFSA) recommends that infant formula should contain LA and ALA equivalent to 4.5–10.8% and 0.5–0.9% of the energy content.[117] These are precursors of LC-PUFAs such as arachidonic acid and DHA.[118]

Linoleic acid, ALA, and ARA have been recognized as "essential fatty acids (EFAs)" as these cannot be synthesized endogenously and therefore, need to be acquired from diet in adequate amounts (Fig. 1).[118] These FAs cannot be synthesized because the desaturases needed for introducing a double bond at carbons 3 and 6 (counted from the methyl end) are not expressed.[8] Linoleic acid provides nearly 10% of the calories derived from the lipid fraction.[98] In preterm infants, biochemical evidence of EFA deficiency can be detected as early as 72 hours after birth.[119]

Long-chain Polyunsaturated FAs in HM

Increasing data now show that the capacity of young infants to synthesize LC-PUFAs might also be limited.[120] Hence, there may be a justification to supplement infant formulas with LC-PUFAs to bring the levels to resemble HM.[121] The most frequently seen LC-PUFAs are shown in Figure 2. Maternal diet can alter the EFA and LC-PUFA content in HM. Several different elongases and desaturases transform EFAs to LC-PUFAs, such as arachidonic acid, DHA, and EPA, which then serve as substrates for bioactive metabolites such as eicosanoids, lipoxins, resolvins, and protectins.[122,123] Fatty acids are also potential substrates for many elongases and desaturases for the formation of downstream metabolites.[124] In addition to these short-term changes, dietary changes can also change the lipids stored in her adipose tissues and consequently change the lipid composition of her milk.[125] Studies have shown a high correlation between the mother's fish intake and the DHA levels in her plasma and breast milk.[48] Therefore, maternal diet should receive special attention in lactating mothers.

There are some data that show a possible need for supplementing infant formula with monounsaturated FAs such as oleic (C18:1n-9) and palmitoleic acid (C16:1n-7, a product of palmitic acid metabolism).[98] These are normally secreted in HM in adequate amounts.[36] Oleic acid lowers the melting point of TAGs to enhance liquidity required for the formation, transport, and metabolism of milk fat.[126]

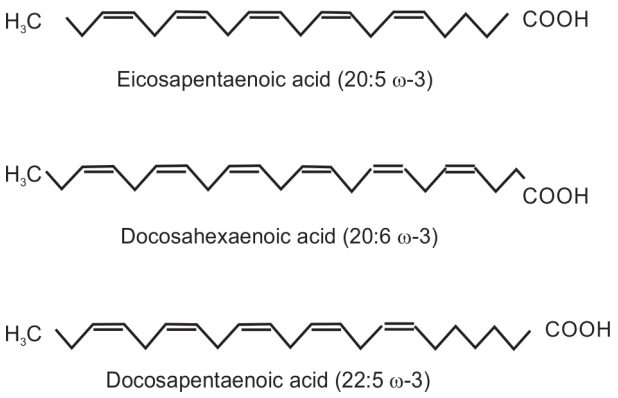


Fig. 3: The most frequently seen LC-PUFAs in human milk

Long-chain polyunsaturated FAs in HM may be derived from diet or drawn from FA storage pools in her own adipose tissue or the liver[107,127] (Fig. 3). Dietary EFA and ω-3 FAs are also known to influence the LC-PUFA content in HM.[36] However, the effects of diet are relatively transient compared with the longer-term changes seen with FAs drawn from body's internal pools.[128] Fatty acids that are synthesized, elongated, or desaturated in the liver or peripheral tissues are more likely to be incorporated into milk fat.[128] Most studies on these changes have focused on the linoleic acid, α-linolenic acid EFAs. A review of 65 studies showed lower variation of ARA compared with DHA between populations with different dietary habits.[129]

The perinatal period is a period of intensive growth of the brain, which contains large amounts of ARA and DHA.[130] There is a likely need for LC-PUFAs in larger amounts for the development of neurological and cognitive functions.[131] Long-chain polyunsaturated FAs are also involved in inflammatory and immunological processes, suggesting a need in optimal maturation of the immune system.[132–134] Long-chain polyunsaturated FAs might also influence adipocyte differentiation and consequently, the risk of obesity in childhood or later.[120]

Carnitine promotes the transport of LC-PUFAs into the mitochondria for oxidation and removal of short-chain FAs that accumulate in mitochondria.[135] Preterm infants are at risk for relative deficiency of carnitine because of their dependence on lipids as an energy source and limited endogenous synthetic ability, although the clinical need is still unproven.[136] Carnitine is detectable in HM and is currently added to standard term and preterm formulas.[137] In preterm infants not receiving supplemental carnitine, plasma and tissue carnitine levels may fall even in the presence of adequate precursor amino acid concentrations.[136,138]

Yaron et al.[139] showed that infants fed with a formula supplemented with β-16:0 for 6 weeks showed more *Lactobacillus* and *Bifidobacterium* genera in their stools than a control group that received formula containing vegetable-sourced 16:0. The mechanisms are unclear, but there is a possibility that the position of palmitic acid on the TAGs may have influenced that gut microbiome.[140] Current infant formulas typically include palm, coconut, soybean, and sunflower oils as the primary lipid sources.[98] The development of structured lipids that resemble HM fat may improve the safety of formula feedings.[73]

The synthesis for LC-PUFAs in HM has severe unique patterns. Similar to the desaturases and elongases seen in plasma, red blood cells, and the adipose tissue, the efficacy of similar enzymes seen in HM in conversion of EFAs into LC-PUFAs may determine the relative concentrations of various FAs in milk.[132] However, there may be subtle differences between individual FAs. Alpha-linolenic acid, the precursor of the n-3 series LC-PUFAs, is processed differently than LA.[36] Nearly 65% of milk ALA seems to be directly derived from maternal diet, not from the body stores.[36,141] The association of maternal DHA intake (fish oil or other sources of ω-3 LC-PUFAs) with milk DHA levels is stronger than that of maternal ARA intake.[142] There are a few studies that tested the effects of n-3 supplementation on milk fat composition in combination with ARA supplementation.[36] In a Dutch study, 88 breastfeeding women received DHA (220 mg/day), with milk samples collected in the second and 12th week of lactation.[143,144] Docosahexaenoic acid percentages were significantly higher than in the placebo group. In the supplemented group, the addition of ARA led to higher median ARA percentages in milk. As the supplementation had started already mid-pregnancy, the findings suggested that increased ARA excretion with milk could be the result of increased direct transfer from the diet and increased ARA contribution to the flux of fatty acids into milk passing maternal storage pools. Several other studies have shown similar findings.[145–147] There is a need to re-evaluate current beliefs that only 10% of milk ARA is directly derived from the diet.

DHA in HM

Docosahexaenoic acid is an important LC-PUFA in HM, most of it *is* derived from its precursor, α-linolenic acid (18:3 ω-3).[148,149] It is highly enriched in the brain and is important for neurodevelopment.[131] Infants, particularly those born preterm, can develop LC-PUFA deficiencies because of their limited ability to synthesize these FAs.[110] Here, an important adaptation in the mother–infant axis is that preterm milk contains more C8–C14 FAs and LC-PUFAs than term milk, and the LC-PUFA content gradually decreases with increasing postnatal age.[115] These developmental adaptations may be helpful as shorter FAs might be easier to digest, and LC-PUFAs are important for brain and retinal development.[80]

Docosahexaenoic acid is important for neurologic development and advancement of visual acuity.[116] Lipids are an important constituent of brain matter, and DHA constitutes 30–40% of the fatty acids in the gray matter and the synaptic membranes.[150] The retina, particularly its outer rod segment, also has a high DHA content.[151] Fatty acids are known to alter gene expression in the developing brain, and hence, are likely to alter long-term neurodevelopment and metabolism.[152] These effects are most likely to be seen in the fetus/premature infants, who may not yet have the capacity to absorb/synthesize specific FAs such as the LC-PUFAs.[153]

Western diets may not always provide appropriate amounts of DHA for lactating mothers, and DHA levels may fall by 40% in preterm infants during the first week after birth.[154] Many studies have measured the effects of DHA supplementation in lactating mothers on the FA composition in HM and infant plasma.[113,155–157] Maternal daily supplementation of 1200 mg of DHA can increase HM and infant plasma DHA concentrations to almost 12 and 2–3 times higher than in controls.[158] These higher levels remained elevated even after 6 weeks of maternal supplementation of DHA at 400 mg/day.[159,160] Current evidence suggests that DHA supplementation to lactating mothers is safe and effective in increasing DHA levels in HM.[161]

Some infant formulas are now being supplemented with these FAs.[162,163] In preterm infants, raising the DHA levels by 2–3 times may have improved neurodevelopmental and cognitive outcomes such as information processing.[164,165] In term infants, LC-PUFA supplementation may improve visual acuity by 12 months of age.[166] Cholesterol is another small (9–12 mg/dL), but an important lipid constituent of HM.[167] Breastfed infants may receive more cholesterol per kg body weight in feedings than adults but have better lipid profiles.[168] There is a possibility that early breastfeeding associated with high measured total blood serum cholesterol may prevent, not raise, some of the risks of developing cardiovascular diseases later in life.[169]

In preterm infants, DHA and its downstream metabolites, the oxylipins, are important regulators of inflammatory responses.[170] The deficiency of DHA after birth can augment inflammation, particularly in preterm infants.[171,172] Infants with higher mean DHA levels may be less likely to develop chronic lung disease (CLD).[173] Docosahexaenoic acid supplementation may also be protective against necrotizing enterocolitis (NEC).[174] The mechanisms by which DHA supplementation to lactating mothers or to infants attenuates inflammation are still not clear; one possibility is that N-docosahexaenoylethanolamine (synaptamide), a neurogenic and synaptogenic metabolite of DHA, may mediate some of these anti-inflammatory effects.[175,176] Maternal diet high in ω-3 fatty acids upregulates genes involved in neurotrophin signaling in fetal brain during pregnancy in C57BL/6 mice.[177] The mechanisms of DHA effects in humans are not clear.

Docosahexaenoic acid and other LC-PUFAs can be acquired in diet, but humans can also synthesize some small quantities from precursors linoleic acid (18:2 ω-6) and α-linolenic acid (18:3 ω-3).[107] The most important pathways include Δ4-desaturation, β-oxidation, and carbon recycling.[178] The FA desaturase (FADS) gene cluster consists of a family of three genes located on human chromosome 11q12-13.1 that yields enzymes catalyzing the insertion of double bonds in PUFA, monounsaturated FAs and palmitic acid.[179] FADS2 can code for pathways involving Δ6-, Δ8-, and Δ4-desaturation.[180] FADS1 codes for a Δ5-desaturase, leading directly to the signaling precursor ω-6 arachidonic acid (20:4 ω-6) and to ω-3 eicosapentaenoic acid (20:5 ω-3).[162,181] Despite these well-established classical results, substrate competition is known to modulate the relative activity of desaturases that defines total PUFA composition of tissues.[182]

In parenteral nutrition, many lipid preparations now contain DHA and AA.[183,184] However, these preparations may not always correct postnatal FA deficits.[185] Eicosapentaenoic acid levels may be elevated due to the fish oil component in these lipid preparations but the DHA and AA levels may decline.[186–190] Low AA levels could possibly be associated with suboptimal clinical outcomes such as with increased risk of late-onset sepsis and retinopathy of prematurity.[171,191,192]

Docosahexaenoic acid and AA are naturally expressed in HM, and so most infant formulas for term and preterm infants are now supplemented with these FAs.[163] Many RCTs have been conducted to evaluate the addition of DHA and AA to preterm formulas.[109,193] Most studies show positive or no changes in growth, although few show negative effects.[193] Findings of improved visual acuity have been inconsistent. Formula supplemented with DHA and AA seems to improve visual acuity,[166] but the effects on neurodevelopment remain unclear.[193] Current recommendations advocate for VLBW infants to receive 55–60 mg of DHA and 35–45 mg per kg/day of AA.[194,195]

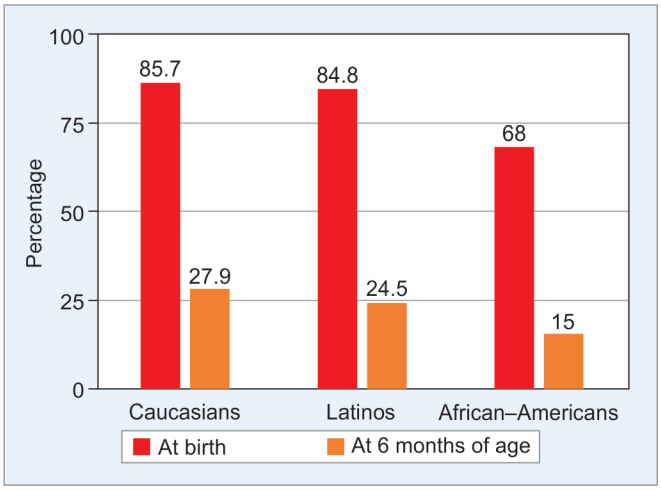


Fig. 4: HM feeding rates by race in the United States (CDC, 2017)

A recent Cochrane review[196] showed that ω-3 LC-PUFA supplementation during pregnancy is effective in reducing the incidence of preterm birth, although it may increase post-term pregnancies. Preterm birth <37 weeks and early preterm birth <34 weeks were reduced in women receiving omega-3 LCPUFA compared with no ω-3.[196] There as a possibility of reduced risk of perinatal death and of neonatal care admission, reduced risk of LBW babies; but a small increased risk of LGA babies with ω-3 LC-PUFAs.[196] For GRADE quality assessments,[197] the conclusions for perinatal outcomes were viewed as high- or moderate-quality evidence.

Conclusion

Human milk is the primary source of nutrients for neonates, and may be the best option from the points of view of nutritional, immunological, food safety, and growth and development.[198] It also improves mother–infant bonding, and facilitates the emotional, cognitive, and nervous system development of the infant.[199] Unfortunately, rapid urbanization and social limitations have been major constraints in promoting HM feedings.[200,201] Worldwide, only 35–40% of infants receive HM from birth to 6 months of age.[202–205] Breastfeeding rates diverge widely along the lines of race, socioeconomic status, and ethnicity (summarized in Figure 4).[206,207]

Despite all the educational and logistical strategies to promote HM feeding, some infants will still continue to need at least some formula feedings because of medical, social, or other reasons.[208] Therefore, there will be a need to develop and improve infant formulas that closely mimic the nutritional and chemical characteristics of HM.[209] Timely delivery of physiologically important lipids will be needed to improve growth and development by optimizing the energy contents, without unduly increasing the osmolar loads.[73,210] We also cannot overemphasize the importance of lipids in neurological development, protection of the gastrointestinal tract, immune defenses, and cholesterol metabolism.[52,154,211,212]

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REVIEW ARTICLE

Prevalence of Gram-negative Bacteria in Maternal Cervical Secretions: A Systematic Review and Meta-analysis

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Received on: 23 November 2022; *Accepted on:* 25 November 2022; *Published on:* 23 December 2022

Abstract

Background: In neonates, early-onset sepsis (EOS) occurring within 72 hours after birth is an important cause of mortality worldwide. Emerging data show that EOS may occur more frequently in tropical and peri-equatorial regions with more gram-negative bacteria than in the Western countries. This systematic review aimed to estimate the prevalence of gram-negative bacteria in the maternal genital tract during the peripartum period.

Materials and methods: We explored the primary research studies that reported the presence of gram-negative bacteria in the maternal genital tract using the software STATA, version 17.1. Five databases, PubMed, Embase, Scopus, Web of Science, and ProQuest were searched until October 2022. Data were analyzed using random-effects meta-analyses to determine the prevalence of gram-negative bacteria in the maternal genital tract.

Results: Fifteen studies qualified for analysis by our predetermined inclusion criteria. The overall prevalence of gram-negative bacteria in cervical secretions was 23.20% (95% CI [confidence interval]: 11.77–37.08, I^2: 99.79%). *Escherichia coli* (15.3%) and *Acinetobacter* (0.36%) species reported the highest and lowest prevalent bacteria, respectively. The prevalence of other gram-negative species was *Klebsiella pneumoniae* (0.47%), *Pseudomonas* (2.81%), *Enterobacter* (3.33%), *Alcaligenes faecalis* (1.32%), *Proteus vulgaris* (10.0%), and *Providencia alcalifaciens* (10%). Most of the studies were from tropical countries, and there was a positive linear relationship between the studies.

Conclusion: Gram-negative colonization of the maternal cervical-vaginal tract may be more frequent than previously recognized in tropical/peri-equatorial regions of the world. Early identification of these bacterial pathogens may help in timely evaluation and treatment of these infants.

Keywords: Critical care devices, Early-onset sepsis, Gram-negative sepsis, Intestinal disorders, Newborn, Premature, Prolonged hospitalization.

Newborn (2022): 10.5005/jp-journals-11002-0051

Key Points

- There are important differences in bacterial pathogens causing EOS in different parts of the world. In the West, gram-positive bacteria such as group B streptococci (GBS) are an important cause. However, in tropical and peri-equatorial regions, gram-negative pathogens are frequently identified in EOS whereas pathogens such as GBS are uncommon.
- In EOS, the pathogenic bacteria identified in maternal cervical–vaginal flora are believed to play an important role.
- We performed a systematic review and meta-analysis of the prevalence of gram-negative bacteria in maternal cervical secretions to (1) determine the relative frequency of these bacteria in these secretions and (2) determine whether there are geographical variations in the maternal genital flora, even if the evidence is limited, to determine the need for future studies.
- Fifteen studies qualified for analysis based on the inclusion criteria. The overall prevalence of gram-negative bacteria was 23.20% (95% CI: 11.77–37.08, I^2: 99.79%). We need focused studies to study the maternal genital flora in tropical and peri-equatorial regions.

Introduction

Neonatal sepsis affects up to 20% of newborn infants and is one of the leading causes of morbidity and mortality in these

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How to cite this article: Padhi BK, Manna S, Pallepogula DR, *et al*. Prevalence of Gram-negative Bacteria in Maternal Cervical Secretions: A Systematic Review and Meta-analysis. Newborn 2022;1(4):397–407.

Source of support: Nil

Conflict of interest: None

patients.[1–5] Neonatal mortality has improved over the last 3 decades, but this progress has been slower than older age groups.[5,6] Deaths in newborn infants contribute greater than 40% of all mortality in children under 5 years of age.[6,7] The Sustainable Development Goals target a reduction of neonatal mortality in all countries to less than 12 deaths per 1,000 live births by 2030.[8]

In EOS, neonates become bacteremia and develop some degree of systemic inflammation within 72 hours following birth.[9,10] These infants likely acquire the bacterial pathogens from the maternal cervical/vaginal secretions during the perinatal period.[9,11,12] And consistent with this possibility, the known predisposing factors associated with EOS include conditions with altered bacterial flora in the birth canal such as during maternal chorioamnionitis or cervical/vaginal colonization with bacteria such as GBS. In other cases, there could be abnormal exposure to various pathogens following procedures such as cervical cerclage and amniocentesis, which can disrupt the amniotic cavity, premature and/or prolonged rupture of membranes, and premature onset of labor.[10,12–19] The infants who get exposed to infectious agent(s) *in utero* or during delivery are at risk of developing sepsis because of their immature immunological responses, and also because they have not had any access to appropriate medical treatment for variable periods of time following the exposure to bacteria prior to delivery.[15,20] The most frequently identified pathogens include gram-positive bacteria such as GBS, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus viridans*, and *Enterococcus* spp., and gram-negative pathogens such as *E. coli*, *Klebsiella* spp., and *Haemophilus influenzae*.[9,21–24]

Emerging data show that the incidence of EOS in tropical and peri-equatorial regions is not only higher than the Western countries with relatively temperate climates, but the spectrum of the pathogenic bacteria may also be different. Sepsis due to gram-negative pathogens, and in some cases, Staphylococci, seems to be more frequent in the tropical and peri-equatorial regions.[25–29] In the West, gram-positive bacteria such as GBS, other Streptococci, and Staphylococci are seen more often than gram-negative bacteria.[26,27,30–32] Gram-negative EOS in tropical and peri-equatorial regions may result in higher mortality not only because of aggressive clinical disease but also because of limitations in health-care infrastructure.[28,33,34] *Escherichia coli* is the most frequently recorded cause of neonatal infections and is known to cause significant morbidity and mortality.[35] Vertical mother-to-infant transmission of *K. pneumoniae* can also cause neonatal sepsis;[36–38] in tropical and peri-equatorial countries, it may account for up to 20% of neonatal sepsis-related mortality.[39]

We currently have limited data to evaluate the possibility that maternal cervical secretions in the tropical and peri-equatorial regions contain more gram-negative pathogens. There is a need to evaluate the possibility that maternal cervical–vaginal mucosa in these regions is colonized differently with more gram-negative bacteria than in the West. To develop prevention strategies and set research priorities, a deeper understanding of the mechanisms by which infections are transmitted to the fetus/newborn infant is essential. In this study, we searched and evaluated existing relevant maternal and neonatal data. A systematic review was performed to assess the proportion of gram-negative bacteria in the cervical secretions of pregnant women.

Materials and Methods

Study Selection and Electronic Search

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) criteria were followed to design this study (Flowchart 1).[40] We conducted a PRISMA-compliant literature search for relevant studies using phrases such as "cervical discharge," "gram-negative bacteria," and "Cervical mucus" in PubMed, Scopus, Web of Science, and EMBASE. The search criteria were broad in order to find all studies that included any health result, which would require a significant number of search phrases to be exhaustive. Appendix 1 details the search strategy.

Studies investigating the prevalence of gram-negative bacterial colonization in pregnant women were included. The reports published in languages other than English were excluded. Case reports and letters to the editor were not considered. Inclusion and exclusion criteria were defined as listed in Appendix 2. Mendeley Desktop was used to enter the search results.[41] DRP and SM evaluated the articles for the relevance of contents, title, and abstract, and the inclusion criteria were then applied to the full-text articles. If there was a disagreement among the reviewers, a discussion was held to reach an agreement; otherwise, help was sought from a third author.

Data Extraction and Management

We adopted a standardized form to evaluate each included study for the first author, year of publication, type of study, sample size, and prevalence rates of gram-negative bacterial infections.

Data Analysis

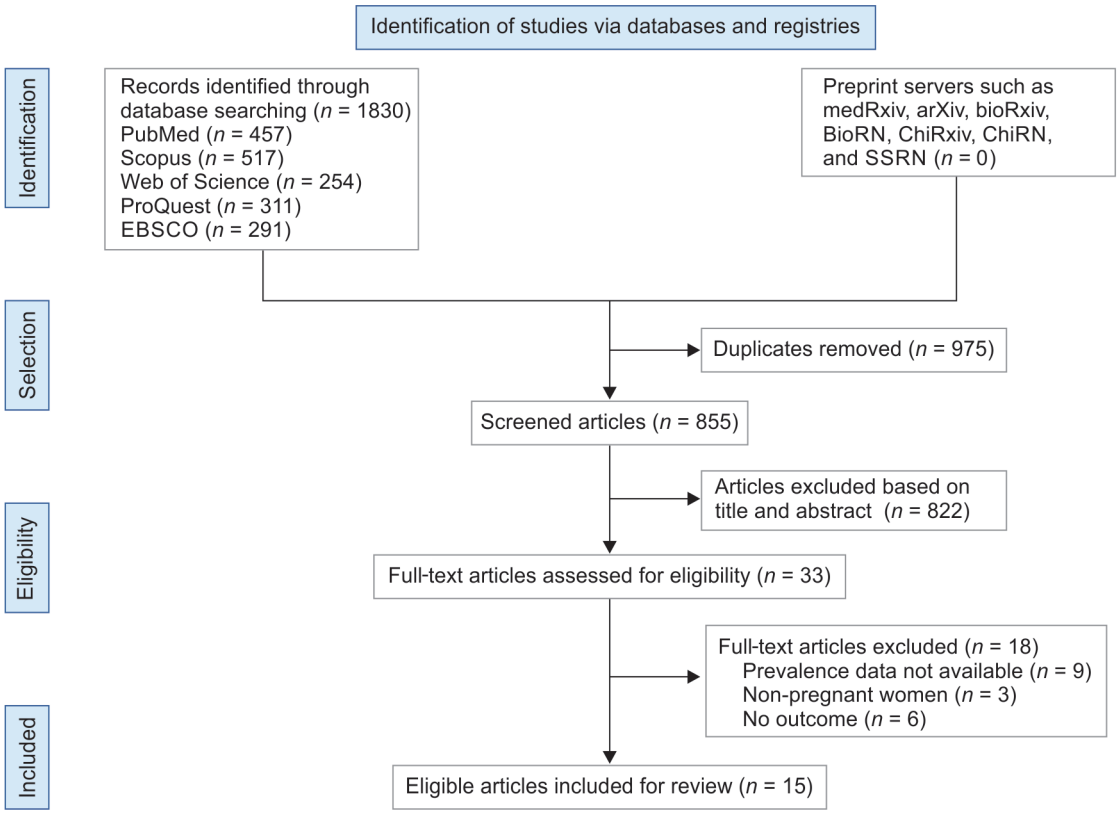
A random-effect meta-analysis[42] was used to group the prevalence of the presence of GNB in the cervical secretion of pregnant women, assuming heterogeneity between studies. To address the issues provided by the proportions near or on the boundaries of 0 and 1, we produced individual sample estimates with 95% CIs.[43] The percentage of variation resulting from heterogeneity was calculated using I^2, with values of more than 75% indicating significant heterogeneity.[44,45] The degree of funnel plot asymmetry was assessed using Egger's test when at least 10 estimates were available.[46] Funnel plots were made to visualize transformed proportions versus their standard errors. All analyses were carried out in STATA (version 17.0).

Quality Assessment

The risk of bias in observational research was evaluated by two authors (BKP and SM) using the Newcastle–Ottawa Scale.[47] Star ratings were assigned to individual quality components such as selection, comparability, and outcome. The collection included four items, and a star was awarded to each item. The comparison only used one item, which scored two stars. Three products, each with one star, were the result. Thus, a single study could have received up to nine stars. Each study was given a certain number of stars. The research was considered to be of good quality if it achieved six or more stars. Any disagreements or ambiguities were resolved by team members coming to an agreement. Appendix 3 depicts the quality of included studies in this review.

We have taken baseline data from three prospective cohort studies (Febriani et al.,[48] Ngonzi et al.,[49] and McDonald et al.) [50] and one randomized controlled trial (Husain et al.).[51] We treated it as a cross-sectional study while performing the quality assessment.

Flowchart 1: PRISMA flowchart for included studies in systematic review and meta-analysis of the prevalence of Gram-negative bacterial infection in maternal cervical secretion



Publication Bias

Egger's meta-regression test[52] and Funnel plots[52] were used to assess the small study effects. The study heterogeneity was reported using the I^2 measure of consistency.[53]

Results

Prevalence of Gram-negative Bacteria

Escherichia coli was the most extensively analyzed gram-negative bacterial species; it was examined in 15 studies.[48,50,54–60] *Klebsiella pneumoniae* was examined in 12.[48,50,54–56,58–62] *Pseudomonas* spp. in 7,[34,48,54,55,58,61] *Enterobacter* spp. in 4,[48,55,56,58] *Acinetobacter* spp. in 3,[48,54,55] and *P. vulgaris* in 4 studies.[48,50,54,55] *Alcaligenes faecalis* and *P. alcalifaciens* were reported in 1 study.[48] Figure 1 shows the overall pooled estimate of the prevalence of gram-negative bacteria in maternal cervical secretions through forest plots. The overall prevalence was found to be 23.20% (95% CI: 11.77–37.08).

Studies that qualified for further analysis in this project showed significant heterogeneity (Fig. 1). We used the DerSimonian and Laird random-effects model[44] to calculate the total pooled prevalence of gram-negative bacteria since it offers more conservative effect sizes. The publication year and study sample size were considered as potentially associated with the variation in prevalence. However, when we performed a univariate meta-regression analysis,[63] neither showed a statistically significant variation (Tables 1 and 2).

Publication Bias

A Funnel plot was used to test publication bias; upon initial inspection, it looked fairly asymmetrical (Fig. 2), indicating the presence of publication bias.[52] However, Egger's and Begg's tests[52] did not show any significant publication bias; the respective *p*-values were 0.0842 and 0.1836, respectively.

Relationship between Studies

We constructed a bubble graph to assess the relationship between studies.[64] A positive linear relationship was seen between studies with a larger sample size, which revealed a high prevalence of gram-negative bacteria (Fig. 3). In these depictions, the size of each bubble is determined by the effect estimated by the prevalence of individual studies. Larger bubbles signify a greater prevalence.

Subgroup Analysis

We performed subgroup analyses based on the type of gram-negative bacteria to evaluate potential sources of heterogeneity. The pooled prevalence of gram-negative bacteria was 23.20% (95% CI: 11.77–37.08, I^2: 99.79%). The highest and lowest prevalent gram-negative bacteria were identified as *E. coli* (15.34%, 95% CI: 6.89, 26.33) and *Acinetobacter* spp. (0.36%, 95% CI: 0, 1.48), respectively.

The prevalence of *K. pneumoniae* was estimated to be 2.65% (95% CI: 0.63, 5.86), 0.47% for *Pseudomonas* spp. (95% CI: 0.00, 1.54), 2.81% for *Enterobacter* spp. (95% CI: 0.59, 6.40), 3.33% for *A. faecalis* (95% CI: 1.14, 9.35), 1.32% for *P. vulgaris* (95% CI: 0.29, 2.94), and 10% (95% CI: 5.35, 17.92) of *Providencia alcalifaciens* (Table 3).

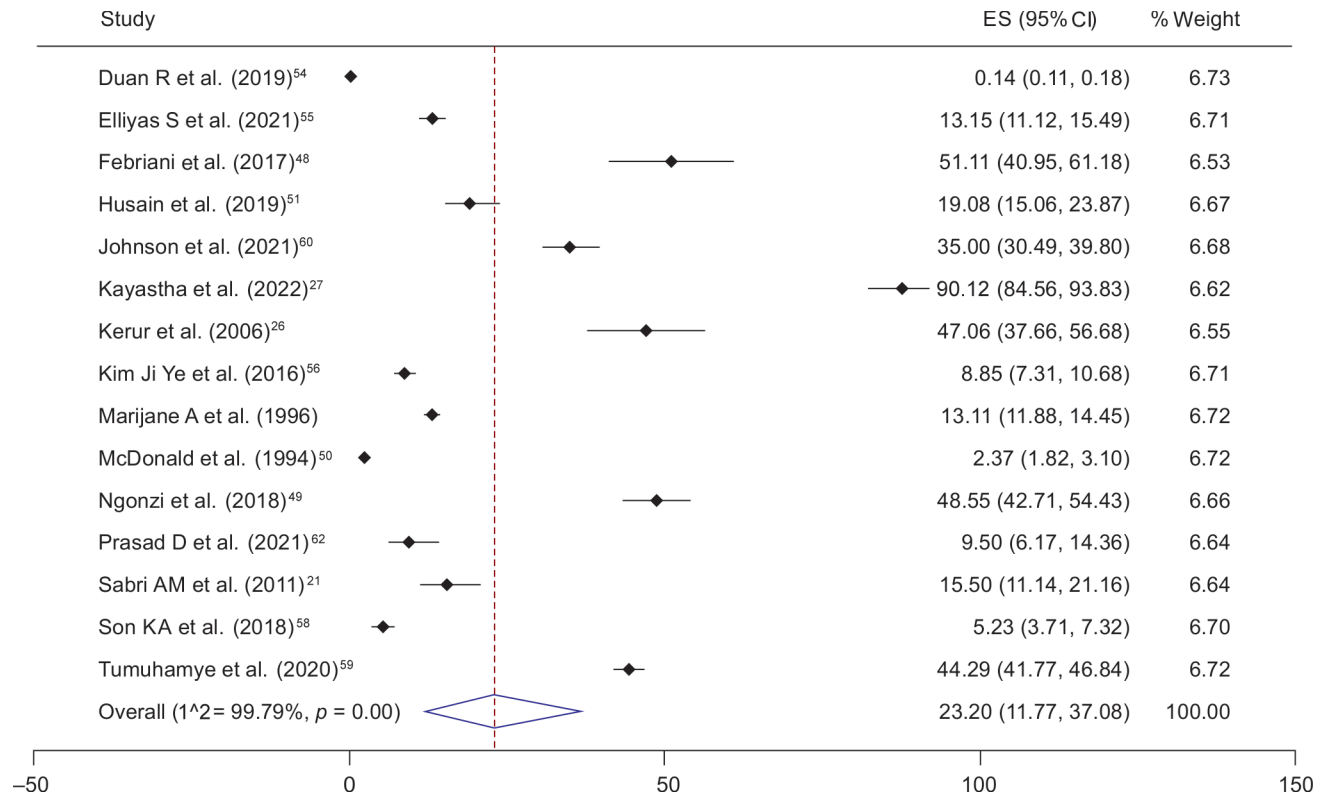


Fig. 1: Forest plot of the pooled magnitude of the prevalence of Gram-negative bacterial infection in maternal cervical secretion

Discussion

This meta-analysis reported a pooled prevalence of gram-negative bacteria of 23.2% in maternal secretions. Most studies reported were from tropical countries. A global systematic review of multidrug-resistant gram-negative bacteria and their transmission to newborn infants identified a pooled proportion of 19% neonates who acquired these infections through mother-to-child transmission.[24]

In vertically transmitted mother-to-infant infections, an intrauterine pathogen can enter the amniotic sac, replicate, and can cause fetal/neonatal sepsis.[65] Transmission can occur during early pregnancy, even if the embryonic membranes remain intact, and infection may persist for a few weeks. Hematogenous infections of the fetus can also arise through placental infection. Furthermore, the transmission of gram-negative bacteria can also occur during delivery when the fetus comes into contact with contaminated vaginal secretions.[66] It is a potentially serious problem because these bacteria are often resistant to antibiotics, causing severe infections that are difficult to treat. In this meta-analysis, we report a pooled prevalence of gram-negative bacteria of 5% among pregnant women. However, the problem might be larger; a global systematic review of multidrug-resistant gram-negative bacteria and their transmission rate to newborns identified a pooled proportion of 19% of the neonates who acquired infection after mother-to-child transmission.[34]

Klebsiella pneumoniae is known to harbor numerous plasmids and can rapidly acquire resistance to multiple antibiotics. These species can survive in the environment and within the human gut, and are known to be one of the commonest reasons for sepsis outbreaks in hospitals in tropical and peri-equatorial regions.[67] *Klebsiella pneumoniae* has been identified as a cause of fetal death as early as 18 weeks of gestation due to acute suppurative placentitis.[68] Cervical–vaginal colonization with *K. pneumoniae* can result in severe infections that invade the uterine cavity, cause chorioamnionitis, preterm delivery, and fetal complications including sepsis, respiratory distress, and patent ductus arteriosus.[69,70]

Escherichia coli is the second most extensively studied species of gram-negative bacteria. A few studies have reported that higher rates of *E. coli* colonization might reflect increased vaginal exams during labor or increased vaginal contamination by anorectal bacteria resulting from prolonged or obstructed labor.[56–58,61] Interrupted labor may be seen in 13–49% mothers. It is known to cause serious illness in infants and lead to various adverse outcomes, including neonatal sepsis, meningitis, and death. *Escherichia coli* infections are a significant cause of infant morbidity and mortality worldwide, and early diagnosis and treatment of *E. coli* infections is essential to improve outcomes.

Pseudomonas aeruginosa seems to cause EOS less frequently, even in infants with identifiable risk factors.[71] However, the mortality rate from *P. aeruginosa* infections in neonates can be as high as 56%. These bacterial species are known to account for approximately 2% of the commensal vaginal flora and, therefore, could have been undetected in vaginal discharge.[72] The pooled prevalence of multidrug-resistant *Pseudomonads* can be high, which can be a cause for concern.[59]

Acinetobacter spp. are the second most common nonfermenting gram-negative pathogens isolated from clinical samples after the Pseudomonads. It is listed by the American Society of Infectious Diseases as one of the six most dangerous microorganisms.[73] The most well-recognized species is *Acinetobacter baumannii*. *Acinetobacter lwoffii*, *Acinetobacter haemolyticus*, and *Acinetobacter*

Table 1: Baseline characteristics of the studies included for meta-analysis

Author (year)	*Country*	*Study design*	*Study population*	*Gram-negative bacteria isolated (n)*
Duan et al. (2019)[54]	China	Cross-sectional	49,496	*Escherichia coli* (*n* = 38) *Klebsiella pneumoniae* (*n* = 4) *Acinetobacter* species (*n* = 17) *Pseudomonas* species (*n* = 10)
Elliyas et al. (2021)[55]	India	Cross-sectional	920	*Escherichia coli* (*n* = 82) *Klebsiella pneumoniae* (*n* = 18) *Enterobacter* spp. (*n* = 15) *Pseudomonas aeruginosa* (*n* = 3) *Acinetobacter* species (*n* = 3)
Febriani et al. (2017)[48]	Indonesia	Prospective cohort	90	*Escherichia coli* (*n* = 3) *Klebsiella pneumoniae* (*n* = 3) *Enterobacter* (*n* = 21) *Proteus vulgaris* (*n* = 3) *Providencia alcalifaciens* (*n* = 9) *Pseudomonas aeruginosa* (*n* = 1) *Alcaligenes faecalis* (*n* = 3) *Acinetobacter* species (*n* = 3)
Husain et al. (2020)[51]	UK	Randomized controlled trial	304	*Escherichia coli* (*n* = 58)
Johnson et al. (2021)[60]	Uganda	Cross-sectional	400	*Klebsiella pneumoniae* (*n* = 52) *Escherichia coli* (*n* = 40) *Pseudomonas aeruginosa* (*n* = 7) *Proteus vulgaris* (*n* = 7)
Kayastha et al. (2022)[27]	Nepal	Cross-sectional	162	*Escherichia coli* (*n* = 146)
Kerur et al. (2006)[26]	India	Cross-sectional	102	*Escherichia coli* (*n* = 39) *Klebsiella pneumoniae* (*n* = 5) *Enterobacter* (*n* = 2) *Pseudomonas aeruginosa* (*n* = 1) *Acinetobacter* species (*n* = 1)
Kim et al. (2016)[56]	Korea	Cross-sectional	1096	*Escherichia coli* (*n* = 63) *Klebsiella pneumoniae* (*n* = 23) *Enterobacter* (*n* = 11)
Krohn et al. (1996)[57]	US	Cross-sectional	2646	*Escherichia coli* (*n* = 347)
McDonald et al. (1994)[50]	Australia	Cohort	2190	*Escherichia coli* (*n* = 52) *Klebsiella pneumoniae* (*n* = 6) *Proteus vulgaris* (*n* = 16)
Ngonzi et al. (2018)[49]	Uganda	Prospective cohort	276	*Escherichia coli* (*n* = 134)
Prasad et al. (2021)[62]	India	Cross-sectional	200	*Escherichia coli* (*n* = 15) *Klebsiella pneumoniae* (*n* = 4)
Sabri et al. (2011)[21]	Iraq	Cross-sectional	200	*Escherichia coli* (*n* = 8) *Klebsiella pneumoniae* (*n* = 4) *Pseudomonas aeruginosa* (*n* = 3)
Son et al. (2018)[58]	Korea	Cross-sectional	593	*Escherichia coli (n = 23)* *Klebsiella pneumoniae (n = 5)* *Pseudomonas aeruginosa (n = 1)* *Enterobacter (n = 1)*
Tumuhamye et al. (2020)[59]	Uganda	Cross-sectional	1472	*Escherichia coli (n = 508)* *Klebsiella pneumoniae (n = 144)*

johnsonii have also been noted in clinical samples.[74,75] Saha et al.[74] reported a similar prevalence of 2.9% of *Acinetobacter* spp. Patients in NICUs are immunocompromised because of prematurity or high severity of illness, which places them at high risk of these infections, particularly with *A. baumannii*.

Gram-negative bacteria can be an important cause of neonatal sepsis in certain geographical and climatic conditions. There is also a high incidence of resistance to empirical first- and second-line antibiotics recommended by the World Health Organization. Further research is needed to determine whether antimicrobial stewardship programs would be adequate to reduce the incidence of these neonatal infections. Additionally, a global commitment is necessary to address the management of gram-negative bacterial infects in pregnant women to reduce vertically transmitted infections to newborn infants.[76]

Recommendations

Given the high prevalence of gram-negative bacteria and the possibility of vertical transmission to the offspring, the risks of fetal/neonatal infections need to be prioritized globally. Many

Table 2: Factors pertaining to the heterogeneity of Gram-negative bacterial prevalence in the current meta-analysis (univariate meta-regression model)

Variables	*Co-efficient*	*p-value*
Publication year	0.0080988	0.256
Sample size	−6.19e-06	0.214

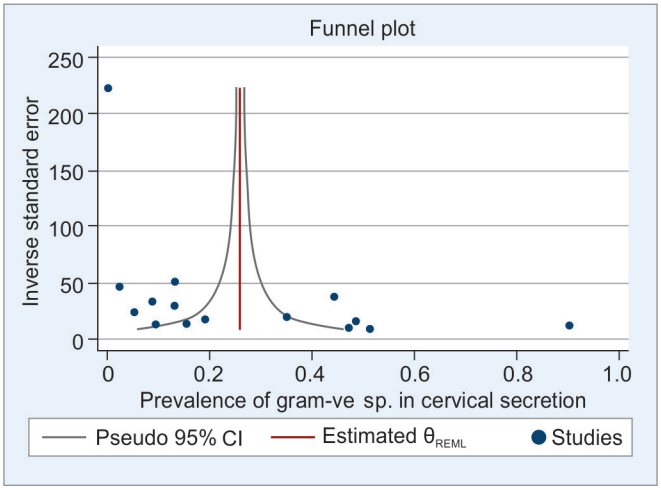


Fig. 2: Funnel plot to assess publication bias of total studies included in the analysis ($n = 15$)

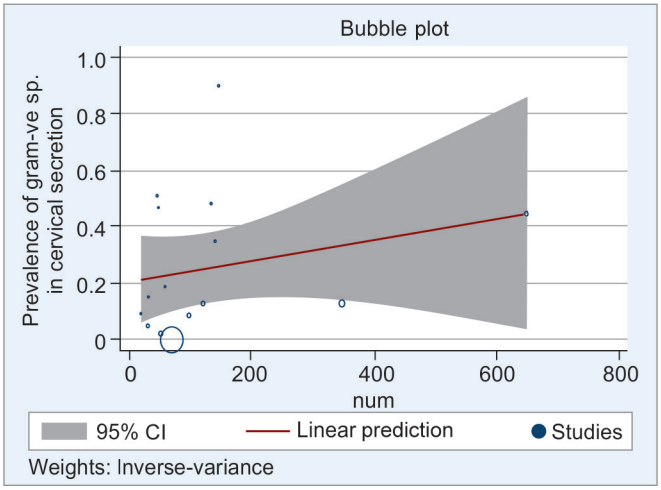


Fig. 3: Bubble plot with 95% CI of pooled prevalence of Gram-negative bacterial infection in maternal cervical secretion

studies have limited these discussions as a problem of low- and middle-income level countries, but the issues might extend beyond economics and may reflect the impact of environmental and climatic conditions. Early detection and appropriate management are essential to efficiently monitor and manage infections among pregnant women and the health of their offspring.

There is a need to develop clear guidelines for early identification of pregnant women with symptoms of bacterial colonization. Further studies are needed to examine the infection patterns in various parts of the globe and deepen our understanding of the pathogenesis. Antimicrobial stewardship programs are obviously important, but we also need large-scale studies to establish the effectiveness of preventive strategies to intervene in the vertical mother-to-child transmission of these bacteria.

Limitations

In our analysis, one clear limitation is the small number of available studies. A larger number of studies are available from the West, which can be a source of bias. Environmental factors might also be an important variable and it might not be prudent to extrapolate conclusions from one region of the world to another without due thought. Socioeconomic status, awareness, demographic characteristics, and even genetic susceptibility may all need to be factored in. Finally, this review incorporates only observational research.

Conclusion

Understanding the causes of neonatal and maternal bacterial infections is very important on a global scale. Improved Identification of the infectious agents seen in pre- and intrapartum periods is important, which requires the easier access to newer microbiological technology. The access to newer technology could potentially facilitate earlier diagnosis and institution of appropriate treatment.

Authors' Contributions

All authors contributed equally to this work.

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Table 3: Subgroup analysis based on the type of Gram-negative bacteria present in maternal cervical secretion

Organisms	*No. of studies*	*Prevalence with 95% CI*	*I^2 (%)*	*p-value*
Escherichia coli	15	15.34% (6.89, 26.33)	98.64	<0.001
Klebsiella pneumoniae	11	2.65 (0.63, 5.86)	56.80	0.02
Proteus vulgaris	3	1.32 (0.29, 2.94)	0.03	0.96
Providencia alcalifaciens	1	10 (5.35, 17.92)	Not applicable	
Pseudomonas sp.	7	0.47 (0.00, 1.54)	0.00	1.00
Acinetobacter sp.	4	0.36 (0.29, 2.94)	1.00	0.99
Alcaligenes faecalis	1	3.33 (1.14, 9.35)	Not applicable	
Enterobacter	5	2.81 (0.59, 6.40)	0.04	0.35

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Appendix 1

The adjusted search terms as per searched electronic databases

Database	*No.*	*Search Query*	*Results*
PubMed	#1	"Gram-negative bacteria" OR "Gram-Negative Bacteria"[Mesh] OR Klebsiella OR "Klebsiella pneumoniae"[Mesh] OR Haemophilus OR "Haemophilus influenzae"[Mesh] OR Shigella OR "Shigella"[Mesh] OR Salmonella OR "Salmonella"[Mesh] OR *Acinetobacter* OR "*Acinetobacter* baumannii"[Mesh] OR "*Acinetobacter* calcoaceticus"[Mesh] OR Citrobacter OR "Citrobacter"[Mesh] OR Neisseria OR "Neisseria meningitidis"[Mesh] OR *Enterobacter* OR "*Enterobacter*"[Mesh] OR "*Enterobacter* aerogenes"[Mesh] OR "*Enterobacter* cloacae"[Mesh] OR Brucella OR "Brucella"[Mesh] OR Pasteurella OR "Pasteurella"[Mesh] OR "Mycoplasma"[Mesh] OR Mycoplasma OR Bacteroides OR "Bacteroides"[Mesh]	943,806
	#2	Pregnan* OR "Pregnancy"[Mesh] OR Trimeste* OR "Pregnancy Trimesters"[Mesh] OR Antenatal OR "Prenatal Care"[Mesh] OR obstetri* OR maternal	1,572,639
	#3	"Cervical Mucus" OR "Cervix mucus" OR "Endocervical secretion" OR "Cervical discharge" OR " Cervical secretions" OR "Cervix Mucus"[Mesh]	4,776
	#4	#1 AND #2 AND #3	457
Scopus			
	#1	(TITLE-ABS-KEY (Gram-Negative Bacteria) OR TITLE-ABS-KEY (Klebsiella) OR TITLE-ABS-KEY (Shigella) OR TITLE-ABS-KEY (Salmonella) OR TITLE-ABS-KEY (*Acinetobacter*) OR TITLE-ABS-KEY (Citrobacter) OR TITLE-ABS-KEY (Neisseria) OR TITLE-ABS-KEY (*Enterobacter*) OR TITLE-ABS-KEY (Brucella) OR TITLE-ABS-KEY (Pasteurella)	
	#2	(TITLE-ABS-KEY (Pregnan*) OR TITLE-ABS-KEY (Trimeste*) OR TITLE-ABS-KEY (maternal) OR TITLE-ABS-KEY (Pregnancy) OR TITLE-ABS-KEY (Antenatal))	
	#3	(TITLE-ABS-KEY (Cervical Mucus) OR TITLE-ABS-KEY (Cervix mucus) OR TITLE-ABS-KEY (Endocervical secretion) OR TITLE-ABS-KEY ("Cervical discharge") OR TITLE-ABS-KEY ("Cervical secretions"))	
	#4	#1 AND #2 AND #3	517
Web of Science			
	#1	Gram-Negative Bacteria (All Fields) or Klebsiella (All Fields) or Shigella (All Fields) or Salmonella (All Fields) or *Acinetobacter* (All Fields) or Citrobacter (All Fields) or Neisseria (All Fields) or *Enterobacter* (All Fields) or Brucella (All Fields) or Pasteurella (All Fields)	
	#2	Pregnancy (All Fields) or Pregnan* (All Fields) or Trimeste* (All Fields) or Maternal (All Fields) or Antenatal (All Fields) or Postnatal (All Fields)	
	#3	Cervical Mucus (All Fields) or Cervi* (All Fields) or Cervix mucus (All Fields) or Cervical discharge (All Fields) or Cervical secretions (All Fields)	
		#1 AND #2 AND #3	254
ProQuest			
	#1	(Gram-Negative Bacteria OR Klebsiella OR Shigella OR Salmonella OR *Acinetobacter* OR Citrobacter OR Neisseria OR *Enterobacter* OR Brucella OR Pasteurella) AND (Pregnancy OR Maternal OR Antenatal OR Postnatal OR Pregnan* OR Trimeste*) AND (Cervical Mucus OR Cervix mucus OR Cervical discharge OR Cervical secretions)	311
EBSCO Host-Academic Search Complete			
	#1	TX Gram-Negative Bacteria OR TX Klebsiella OR TX Shigella OR TX Salmonella OR TX *Acinetobacter* OR TX Citrobacter OR TX Neisseria OR TX *Enterobacter* OR TX Brucella OR TX Pasteurella	
	#2	TX pregnan* or TX gestat* or TX gravid* or TX maternal or TX mother* or TX puerper* or TX antenat*	
	#3	TX Cervical Mucus OR TX Cervix mucus OR TX Cervical discharge OR TX Cervical secretions	
	#4	#1 AND #2 AND #3	291

Appendix 2

Inclusion and exclusion criteria

	Inclusion	*Exclusion*
Participants	• Female • Any trimester of pregnancy • Any age-group	• Nonpregnant women
Disease	• Gram-negative bacteria	• Bacteria causing sexually transmitted infections • Gram-positive bacteria
Outcome	• Proportion of women colonized with Gram-negative bacteria	
Study	Prevalence studies, cross-sectional studies, cohort studies, case–control studies, and surveys English Language Published and unpublished data	Qualitative, policy, opinion, case series, and letter to the editor (if not providing data on desired outcome)

Quality assessment of studies examining the prevalence of Gram-negative bacterial infection in maternal cervical secretion ($N = 15$)

	Newcastle–Ottawa quality assessment scale for cross-sectional studies								
	Selection				*Comparability*	*Outcome*			
Study	*Representativeness of the sample*	*Sample size*	*Nonrespondents*	*Ascertainment of the exposure (risk factor)*	*The subjects in different outcome groups are comparable based on the study design or analysis. Confounding factors are controlled. Maximum: ☆☆*	*Assessment of outcome Maximum: ☆☆*	*Statistical test*	*Score*	*Evidence quality*
Duan et al. (2019)	☆	☆	☆	☆☆	☆	☆	☆	*8*	Low risk of bias
Elliyas et al. (2021)	☆	☆	☆	☆	☆☆	☆☆	☆	*9*	Very low risk of bias
Febriani et al. (2017)	☆	☆	☆	☆	☆	☆☆	☆	*8*	Low risk of bias
Hussain et al. (2019)	☆	☆	☆	☆	☆☆	☆☆	☆	*9*	Very low risk of bias
Johnson et al. (2021)	☆	☆	☆	☆	☆☆	☆☆	☆	*9*	Very low risk of bias
Kayastha et al. (2022)	☆			☆	☆	☆☆	☆	*6*	Low risk of bias
Kerur et al. (2006)	☆	☆	☆	☆	☆	☆☆		*7*	Low risk of bias
Kim et al. (2016)	☆	☆	☆	☆☆	☆☆	☆☆	☆	*10*	Very low risk of bias
Marijane et al. (1996)	☆	☆	☆	☆	☆	☆☆		*7*	Low risk of bias
McDonald et al. (1994)	☆	☆	☆	☆	☆	☆☆		*7*	Low risk of bias
Ngonzi et al. (2018)	☆	☆	☆	☆	☆☆	☆☆	☆	*9*	Very low risk of bias
Prasad et al. (2021)	☆	☆	☆	☆	☆	☆☆	☆	*8*	Low risk of bias
Sabri et al. (2011)	☆	☆	☆	☆	☆	☆☆		*7*	Low risk of bias
Son et al. (2018)	☆	☆	☆	☆☆	☆	☆☆	☆	*9*	Very low risk of bias
Tumuhamye et al., (2020)	☆	☆	☆	☆	☆	☆☆	☆	*8*	Low risk of bias