Tampere University

BIRESHWAR SINHA

Effect of Kangaroo Mother Care in Low Birth Weight Infants on Breastfeeding Performance, Gut Function, and Maternal Depressive Symptoms in Low Middle Income Populations in the Indian Subcontinent

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ABSTRACT

Infants born low birth weight (LBW) have an increased risk of death and illness with many short-term and long-term consequences. As defined by the World Health Organization (WHO), Kangaroo Mother Care (KMC) is an intervention encompassing skin-to-skin-contact between the mother and the infant along with exclusive breastfeeding (1). The WHO recommends practice of KMC in LBW infants. This recommendation is based on the evidence that KMC can substantially reduce deaths and prevent morbidities in LBW infants. Despite the WHO recommendations, there has been reports of resistance towards implementing KMC in developing countries and the estimated coverage of KMC is low. Knowledge on the wide range of benefits of KMC is lacking amongst health care practitioners as well as in the community. Moreover, the potential benefits of KMC to the mothers is unclear, which often presents as a barrier to its promotion and practice.

The PhD studies were therefore designed to assess the effect of promotion and support of KMC on some important biological outcomes i.e., infant breastfeeding performance (Study I), biomarkers of infant gut function (Study II), and maternal postpartum depressive symptoms (Study III). The hypothesis was that KMC can improve infant breastfeeding performance and reduce the risk of maternal postpartum depressive symptoms through enhanced mother-infant bonding and improve infant gut function through reduced pathogen exposure.

The PhD studies were designed as randomized controlled trials embedded within the larger primary KMC trial titled "Impact of Community-initiated Kangaroo Mother Care on Survival of Low Birth Weight Infants" conducted in rural areas of Haryana, North India. Enrolment in the PhD studies were done between April 2017 to March 2018. In the PhD studies, we enrolled stable singleton LBW infants weighing between ≥1500 to ≤2250 grams within 72 hours of birth, born at home, or at hospital with KMC not initiated.

The intervention included promotion and support for early skin-to-skin contact after birth and lactation management to promote exclusive breastfeeding. The intervention delivery team conducted scheduled visits to the homes of the families on 1, 2, 3, 5, 7, 10, 14, 21, and 28 days after birth. During these home visits, practice of skin-to-skin-contact and breastfeeding were observed. The team helped to resolve any difficulties related to KMC practice. Infants in the intervention and control arms

of the trial received standard of care i.e., home-based newborn care visits delivered by the health workers of the government health system.

Outcome assessments in the PhD studies were conducted by independent and trained teams. Infant breastfeeding performance was assessed using the validated 'infant breastfeeding assessment tool' (IBFAT) at the end of the neonatal period. Effective breastfeeding performance was defined by a IBFAT score of more than or equal to 10. For assessment of gut function, infant stool specimens were collected. Concentration of the fecal biomarkers neopterin, myeloperoxidase, and alpha-1-antitrypsin, were assessed using an automated ELISA system at the end of the neonatal period. The validated 'Patient Health Questionnaire-9' (PHQ-9) was used to assess maternal postpartum depressive symptoms at 28 days after birth. Moderate to severe postpartum depressive symptoms was defined by a PHQ-9 score of ≥10.

In Study I, among the 550 enrolled participants, outcome assessments were completed in 98% in the intervention arm and 95% in the control arm. In Study II, among the 200 enrolled participants, assessments of fecal biomarkers were completed in 99% to 100% of the infants in both intervention and control arms. In Study III, among the 1950 enrolled participants, assessments were completed in 93% in the intervention arm, and 94% in the control arm.

In Study I, effective breastfeeding performance was observed in 92% (232/252) of the infants in the intervention arm and 81% (223/276) of the infants in the control arm. The adjusted prevalence ratio (95% CI) for effective breastfeeding performance was 1.24 (1.16 to 1.32), corresponding to an effect of 24% (16 to 32%).

In Study II, between the intervention arm and control arm participants, the adjusted difference in means in the log-transformed concentration of fecal neopterin was 0.03 (95% CI -0.15 to 0.21), myeloperoxidase was 0.28 (95% CI -0.05 to 0.61), and alpha-1-antitrypsin was 0.02 (95% CI -0.30 to 0.34. There was no substantial difference observed in any of the measured fecal biomarkers.

In Study III, moderate-to-severe postpartum depressive symptoms were recorded among 10.8% of the mothers in the intervention arm against 13.6% of the mothers in the control arm. The adjusted relative risk (95% CI) for moderate-to-severe postpartum depressive symptoms was 0.75 (0.59 to 0.96). The corresponding efficacy of the intervention was 25% (4% to 41%).

In conclusion, the findings showed that in low-middle income neighbourhoods in Haryana, North India, promotion and support of KMC in stable LBW infants can substantially improve infant breastfeeding performance, reduce the risk of maternal moderate-to-severe postpartum depressive symptoms, but does not have any substantial effect on the measured fecal biomarkers of infant gut function at the end of the neonatal period. The results support promotion of KMC in public health programs in low-middle income populations in India and in similar South Asian countries. Further research to study the effect of KMC in unstable or very low birth weight infants and its long-term effect on maternal and child health outcomes could be useful.

TIIVISTELMÄ

Matalan syntymäpainon (Low Birth Weight, LBW) omaavilla vastasyntyneillä on lisääntynyt riski kuolemaan ja sairauksiin, joilla on monia lyhyen ja pitkän aikavälin seurauksia. Maailman terveysjärjestön (World Health Organization, WHO) määrittelemänä kenguruhoito (Kangaroo Mother Care, kenguruhoito) on interventio, joka kattaa äidin ja vastasyntyneen välisen ihokontaktin yhdessä yksinomaisen imetyksen kanssa (1). WHO suosittelee kenguruhoidon harjoittamista LBW-vauvoilla. Tämä suositus perustuu näyttöön siitä, että kenguruhoito voi merkittävästi vähentää kuolemia ja ehkäistä sairauksia LBW-vauvoilla. Kenguruhoidon käyttöönotossa on kehitysmaissa kuitenkin raportoitu vastustusta ja sen arvioitu kattavuus on vielä alhainen. Tieto kenguruhoidon laajasta hyödystä puuttuu terveydenhuollon ammattilaisilta sekä yhteisöltä. Kenguruhoidon mahdolliset hyödyt ovat usein epäselviä myös äideille, mikä usein toimii esteenä sen käytön edistämiselle ja toteuttamiselle.

Tämän väitöskirjatutkimuksessa arvioitiin kenguruhoidon edistämisen ja tuen vaikutusta tärkeisiin biologisiin päätetapahtumiin, kuten vastasyntyneen rintaruokintakäyttäytymiseen (Tutkimus I), vastasyntyneen suolistofunktion biomarkkereihin (Tutkimus II) ja äidin synnytyksenjälkeisiin masennusoireisiin (Tutkimus III). Hypoteesi oli, että kenguruhoito voi parantaa vastasyntyneen rintaruokintakäyttäytymistä ja vähentää äidin synnytyksenjälkeisten masennusoireiden riskiä parantamalla äidin ja vastasyntyneen välistä vuorovaikutusta sekä edistämällä vastasyntyneen suolistofunktiota ja vähentämällä lapsen altistumista patogeeneille.

Väitöskirjatutkimukset suunniteltiin osaksi suurempaa kliinistä hoitokoetta, jossa testattiin voiko kotona aloitetulla kenguruhoidon vähentää vastasyntyneitten kuolleisuutta ja muutoin edistää heidän ja heidän äitiensä terveyttä. Tutkimus toteutettiin Haryanan maaseutualueilla Pohjois-Intiassa, huhtikuun 2017 ja maaliskuun 2018 välisenä aikana. Tutkimuksen osallistujat olivat hyväkuntoisia, yksisikiöisestä rastaudesta syntyneitä vastasyntyneitä, joiden syntymäpaino oli 1500–2250 grammaa, joille ei aiemmin oltu aloitettu kenguruhoitoa.

Interventio sisälsi varhaisen ihokontaktin edistämisen syntymän jälkeen ja imetyksen tukemisen yksinomaisen rintaruokinnan edistämiseksi. Interventiotiimi teki kotikäynnin interventioryhmän perheiden luokse 1, 2, 3, 5, 7, 10, 14, 21 ja 28 päivää

syntymän jälkeen. Näillä kotikäynneillä tarkkailtiin ihokontaktin ja imetyksen harjoittamista, ja tiimi auttoi äitiä ratkaisemaan mahdolliset vaikeudet kenguruhoidon toteutuksessa. Kontrolliperheille ei tehty näitä kotikäyntejä. Sekä interventio- ja kontrolliryhmien vastasyntyneille tarjottiin tavanomaiset perusterveydenhuollon palvelut, mukaan lukien kotona tapahtuvat vastasyntyneiden hoitokäynnit, jotka kuuluivat julkisen terveysjärjestelmän terveydenhuollon työntekijöiden tehtäviksi.

Tutkimuksen päätetapahtumien arviointi tapahtui itsenäisten ja koulutettujen tiimien toimesta. Vauvojen rintaruokintakäyttäytymistä arvioitiin käyttämällä validoitua "vastasyntyneen imetyksen arviointityökalua" (IBFAT) vastasyntyneisyyskauden lopussa. Tehokas rintaruokintakäyttäytyminen määriteltiin IBFAT-pistemääräksi, joka oli suurempi tai yhtä suuri kuin 10. Suoliston toiminnan arvioimiseksi kerättiin vastasyntyneiden ulostenäytteitä. Ulosteen biomarkkereiden, kuten neopteriinin, myeloperoksidaasin ja alfa-1-antitrypsiinin pitoisuudet, arvioitiin automatisoidulla ELISA-järjestelmällä vastasyntyneisyyskauden lopussa. Äidin synnytyksenjälkeisten masennusoireiden arvioimiseen käytettiin validoitua "Potilaan terveyskyselylomaketta-9" (PHQ-9) 28 päivän kuluttua synnytyksestä. Kohtalaiset tai vaikeat synnytyksenjälkeiset masennusoireet määriteltiin PHQ-9-pistemääräksi, joka oli suurempi tai yhtä suuri kuin 10.

Tutkimuksessa I oli 550 osallistujaa, joista tiedot päätetapahtumista olivat käytössä 98 %:ssa interventioryhmästä ja 95 %:ssa kontrolliryhmästä. Tutkimuksessa II oli 200 osallistujaa ja heistä ulosteen biomarkkereiden arvioinnit suoritettiin 99 - 100 %:lle sekä interventio- että kontrolliryhmässä. Tutkimuksessa III oli 1950 äitiä ja heistä tiedot masennusoireista selvitettiin 93 %:lta interventioryhmässä ja 94 %:lta kontrolliryhmässä.

Tutkimuksessa I tehokas rintaruokintakäyttäytyminen havaittiin 92 %:lla (232/252) interventioryhmän vastasyntyneistä ja 81 %:lla (223/276) kontrolliryhmän vastasyntyneistä. Mahdollisten sekottavien tekijöiden suhteen vakioitu esiintyvyyssuhde (95 %:n luottamusväli) tehokkaalle rintaruokintakäyttäytymiselle oli 1,24 (1,16 - 1,32), mikä vastaa 24 %: vaikutusta (16 - 32 %).

Tutkimuksessa II interventio- ja kontrolliryhmän osallistujien välillä korjattu ero logaritmisesti muunnetussa neopteriinin pitoisuudessa oli 0,03 (95 %:n luottamusväli -0,15 - +0,21), myeloperoksidaasissa 0,28 (95 %:n luottamusväli -0,05 - 0,61) ja alfa-1-antitrypsiinissä 0,02 (95 %:n luottamusväli -0,30 - 0,34). Ulosten biomarkkerien

pitoisuuksissa ei siis havaittu tilastollisesti merkitseviä eroja interventioryhmän ja kontrolliryhmän lasten välillä.

Tutkimuksessa III kohtalaisia tai vaikeita synnytyksenjälkeisiä masennusoireita kirjattiin 10,8 %:lla äideistä interventioryhmässä ja 13,6 %:lla äideistä kontrolliryhmässä. Vakioitu suhteellinen riski (95 %:n luottamusväli) kohtalaisille ja vaikeille synnytyksenjälkeisille masennusoireille oli interventioryhmän äideillä 0,75 (0,59 - 0,96). Tästä laskettuna interventio vähensi keskivaikeiden tai vaikeuiden masennusoireitten esiintyvyyttä 25 % (4 % - 41 %).

Tulokset osoittivat, että Haryanan Pohjois-Intian alueen matalan keskitulotason alueilla kenguruhoidon edistäminen ja tukeminen hyväkuntoisten matalan syntymäpainon omaavien vauvojen kohdalla voi merkittävästi parantaa vastasyntyneiden rintaruokintakäyttäytymistä, vähentää äitien synnytyksenjälkeisten kohtalaisten ja vaikeiden masennusoireiden riskiä, mutta sillä ei ole merkittävää vaikutusta valittujen biomarkkeireiden pitoisuuksiin lasten ulosteessa vastasyntyneisyyskauden lopussa. Tulokset tukevat kenguruhoidon edistämistä julkisen terveydenhuollon ohjelmissa Intian matalan keskitulotason väestöissä ja vastaavissa Etelä-Aasian maissa. Jatkossa olisi hyvä selvittää kenguruhoidon teho sairailla tai erittäin matalan syntymäpainon omaavilla vastasyntyneillä sekä sen pitkäaikaisvaikutukset sekä lasten että äitien terveyteen.

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ABBREVIATIONS

A1AT: Alpha-1-antitrypsin
ANM: Auxiliary nurse midwife
ASHA: Accredited social health activists
CHRD, SAS: Centre for Health Research and Development, Society for Applied Studies
CI: Confidence Interval
CRL SAS: Clinical and Research Laboratories, Society for Applied Studies
DMC: Data management centre
EBF: Exclusive breastfeeding
EE: Environmental Enteropathy
EED: Environmental enteric dysfunction
GLM: Generalized linear models
HBNC: Home-based newborn care
IBFAT: Infant breastfeeding assessment tool
IQR: Inter quartile range
KMC: Kangaroo Mother Care
LBW: Low birth weight
LMIC: Low-middle income country
MD: Mean difference
PHC: Primary health center
PhD: Doctor of Philosophy
PHQ-9: Patient health questionnaire-9
PPD: Postpartum depression
PR or aPR: Prevalence ratio or adjusted prevalence ratio
RR or aRR: Relative risk or adjusted relative risk
SD: Standard deviation
SSC: Skin-to-skin-contact
USG: Ultrasonography
WASH: Water, sanitation, and hygiene
WHO: World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on three original research articles listed below

Publication I: Sinha B, Sommerfelt H, Ashorn P, Mazumder S, Taneja S, Bahl R, et al. Effect of Community-Initiated Kangaroo Mother Care on Breastfeeding Performance in Low Birthweight Infants: A Randomized Clinical Trial. Maternal & child nutrition. 2022;18(4):e13419.

Publication II: Sinha B, Sommerfelt H, Ashorn P, Mazumder S, More D, Taneja S, et al. Effect of Community-Initiated Kangaroo Mother Care on Fecal Biomarkers of Gut Function in Low Birth Weight Infants in North India: A Randomized Clinical Trial. The American journal of tropical medicine and hygiene. 2021;106(3):945-52.

Publication III: Sinha B, Sommerfelt H, Ashorn P, Mazumder S, Taneja S, More D, et al. Effect of Community-Initiated Kangaroo Mother Care on Postpartum Depressive Symptoms and Stress Among Mothers of Low-Birth-Weight Infants: A Randomized Clinical Trial. JAMA network open. 2021;4(4):e216040.

AUTHOR CONTRIBUTIONS

The author of this thesis was the first author of all the three publications.

For all the three publications i.e., Publication I, II and III: The author was involved in conceptualization and design, funding acquisition, data collection and statistical analysis, writing the first draft of the manuscript and finalization of manuscript. The author has full access to the data for all the three publications and take responsibility for the integrity of data and accuracy of the data analysis.

1 INTRODUCTION

Low birth weight (LBW) is a recognised public health problem worldwide. In 2015, the estimated global prevalence of LBW babies (<2500 grams) was 15%, with 20.5 million infants born LBW, of which, 91% was contributed by low- and middle-income countries (2, 3). The World Health Organization (WHO) has targeted a 30% reduction in the prevalence of infants born LBW between 2012 and 2025 (4). In India, 7.8 million babies (or 20% of the country's live births) are born LBW which accounts for around 40% of all the babies born LBW worldwide (3, 5). The infants born LBW have a reduced survival probability and contribute to more than half of the overall neonatal mortality (6). The major determinants of high mortality in these children are due to complications related to prematurity, infections, and poor nutrition (7). Infants born LBW are also at increased risk of stunting and poor neurodevelopment (8, 9). Early interventions to promote better nutrition and prevent infections are crucial to reduce neonatal mortality and improve thriving in the LBW babies.

Kangaroo Mother Care (KMC) is an intervention comprising of skin-to-skin-contact (SSC) between the mother (or other caregiver) and the infant, and exclusive breastfeeding (1). The WHO, in its 2003 guidelines recommends early, continuous, and prolonged KMC practice to be initiated in hospital and can be continued at home KMC for LBW or preterm infants (<37 weeks gestational age) (1). The recommendation was based on evidence from hospital-based studies on KMC, mostly in developed countries, that demonstrated reduction in deaths and illness in LBW infants (10). Despite the WHO recommendation in 2003, there has been reports of resistance towards implementing KMC in developing countries (11). Most countries including India (12) have a policy for KMC provision in LBW babies, yet the estimated coverage of KMC is low and it tends to vary across different levels of health facilities with poorer coverage in lower-level health facilities (13). WHO has published its new recommendation on KMC in 2022 which is discussed later (14).

In 2015-16, data from the National Family Health Survey showed that around one-fifth of all the deliveries in India occur at home (15). The LBW infants born at home or those discharged early miss-out on the KMC intervention that can potentially save lives. Findings from a recent randomized controlled trial among 8402 LBW infants in Haryana, India, showed that promotion of community-initiated KMC was associated with 30% reduction in neonatal mortality (16). In addition, KMC promotion reduced the risk of possible serious bacterial infection, diarrhea, and severe wasting during the neonatal period, i.e., the first 28 days of life (16). However, the potential pathways of the observed clinical benefits of KMC remain largely unclear.

Knowledge on the wide range of benefits of KMC is lacking amongst health care practitioners as well as in the community (17). Some health care practitioners perceive that the survival benefit of KMC is possibly due to closer attention to the child and hypothermia-prevention and may have limited value where incubators are available (17). I hypothesize that the substantially large clinical benefits of KMC in LBW babies may operate through several pathways involving many intermediary biological outcomes beyond preventing hypothermia. Some plausible biological outcomes through which KMC manifests its clinical benefits may be establishment of effective breastfeeding, improvement in breast milk output and better infant milk intake, reduction infant gut inflammation and permeability, and reduction in the risk of maternal postpartum depressive symptoms.

Evidence from previous non-randomized and observational studies suggest that early SSC between the mother and the infant may promote improved attachment to the breast due to better mother-infant bonding, leading to effective breastfeeding performance (18-20). Given that in KMC position, LBW infants are placed in a protective environment and exclusively breastfed, the likelihood of pathogen exposure and clinical infection may be reduced (21) which in-turn might reduce infant gut inflammation and permeability. KMC through better mother-infant bonding and possibly via the release of maternal oxytocin and lowering of cortisol secretion could potentially reduce the risk of maternal postpartum depressive symptoms (18, 22, 23) which in turn may lead to better care of the baby. Although some evidence exists, better-quality evidence is needed to improve knowledge on the biological benefits of KMC.

The aim of the studies included in the PhD thesis was to assess the effect of promotion and support of KMC on infant breastfeeding performance, infant gut inflammation and permeability, and maternal postpartum depressive symptoms, which are important biological outcomes related to infection prevention and nutrition promotion. The new knowledge from this research on the important biological outcomes can be valuable to explain the clinical impact of KMC on infant nutrition, infection prevention, and survival; although causality of the associations may be difficult to establish.

2 REVIEW OF LITERATURE

2.1 Approach

The purpose of the presented literature review is threefold. First, to provide background information on the burden of low birth weight (LBW), understand why it is considered as a public health problem and what are the health issues associated with it. Second, is to describe the history and context of kangaroo mother care (KMC) as an intervention to improve health outcomes in LBW infants. Here I have summarized previous literature on the effect of KMC on clinical health outcomes, issues and perceptions around initiation and practice of KMC. Third, based on available literature I have summarized the possible pathways of effect of how KMC works. Finally, the review identifies the key knowledge gaps and theoretical framework which forms the basis of the PhD studies.

To search for relevant published literature, I searched PubMed®, Cochrane Library and Google Scholar (Table 1). Different keywords were used for the search in various permutation and combinations e.g. [#1 AND #2 AND #3], [#1 AND #2 AND #4], [#1 AND #2 AND #5]. The search was updated till December 2019, which is up to the time when data analysis was initiated for the PhD project. I had also screened the references of the selected articles to find additional relevant articles. To collate relevant information on National and International policies related to KMC and care of LBW infants I searched the websites of national (India) and international health agencies (WHO, UNICEF).

Table 1. Search strategy for review of literature

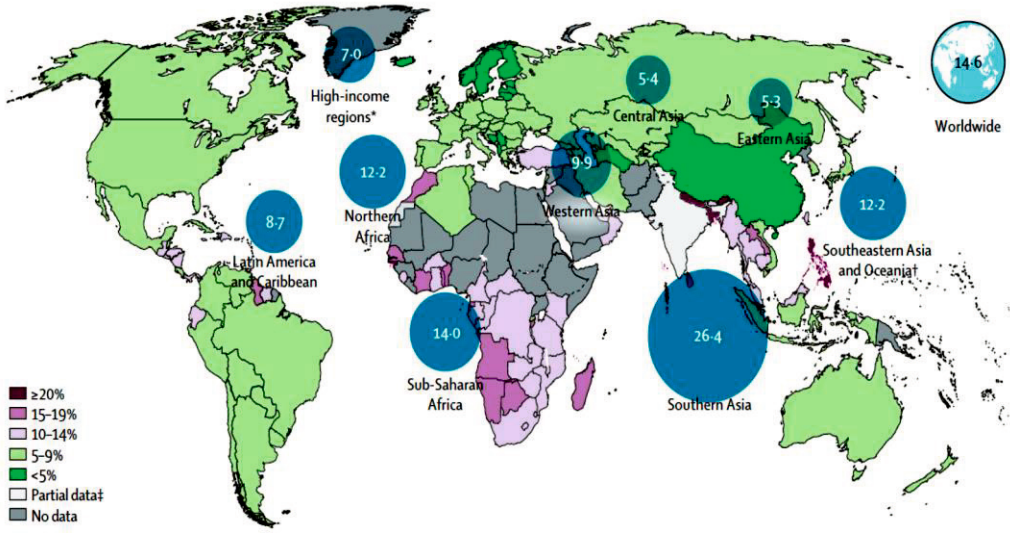
Search No.	Domain	Search Terms
#1	Population	"Low birth weight" Or "Preterm" Or "Premature" Or "Infant"
#2	Exposure or Intervention	kangaroo mother care" Or "kangaroo care" Or "kangaroo mother care method" Or "skin-to-skin contact
#3	Clinical outcomes	"Mortality" Or "Survival" Or "Death" Or "Morbidity" Or "Infections" Or "Sepsis" Or "Hospitalization" Or "Growth"
#4	Biological outcomes	"Hypothermia" Or "Physiological parameters" Or "Vital parameters" Or "Breastfeeding performance" Or "Breastfeeding sufficiency" Or "Gut inflammation" Or "Gut function" Or "microbiome" Or "Stress" Or "Depression"
#5	Issues related to implementation	"Barriers" Or "Enablers" Or "Facilitators" Or "Challenges" Or "Resistance Or "Implementation" Or "Initiation"

2.2 Low Birth Weight: a Public Health Problem

Size at birth is an important indicator of neonatal, post-neonatal health and is associated with longer term health outcomes in adulthood. As per the WHO, LBW is defined as birth weight <2500 g, irrespective of gestational age (4, 24). LBW is a well-established, globally accepted, easy to measure indicator of newborn vulnerability, especially useful in settings where reliable assessment of gestational age is unavailable. LBW may include infants born preterm <37 completed weeks of gestation, or term infants (≥37 weeks) who are born small for gestational age (<10th centile of weight for gestational age and sex) and a very small proportion who are neither (4). It is estimated that of the 140 million live births globally in 2015, 20.5 million births i.e., around 15% were born LBW (3).

Around 90% of the LBW births worldwide are contributed by low- and middle-income countries, mainly south Asia (48%) and sub-Saharan Africa (24%) (3). South Asia is estimated to have the highest prevalence of LBW with 26% of all live births born LBW (Figure 1) (3). India alone accounts for around 40% of all LBW babies globally with around 7.8 million babies (or 20% of the country's total live births) being born with a birth weight less than 2500 grams (25).

Figure 1. Prevalence of Low Birth Weight in Different Regions Across the Globe



Source : Blencowe H et al. Lancet Glob Health 2019;7:e849-60 (3). Permission for reuse from Elsevier based on CC BY 4.0 license.

LBW is a global public health concern and has both short-term and long-term health consequences. LBW infants have an increased risk of death and contribute to more than half (55%) of the overall neonatal mortality (6, 25, 26). The major determinants of high mortality in the infants born LBW are hypothesized to be hypothermia, infections, and poor nutrition. The LBW infants are at higher risk of morbidities including infections (diarrhoea, pneumonia) (27, 28) and poor nutrition with around three-fold higher odds of wasting, stunting, and underweight compared to infants with birth weight >2500 g (8). It is reported that the infants born LBW are at increased risk of developmental delay in childhood (29, 30), motor and cognitive problems in school age to adolescence (30, 31). LBW infants are also at increased risk of developing chronic diseases like diabetes, obesity later in life (32). Moreover, it is observed that the birth weight phenotype can have intergenerational transmission with increased risk of LBW if parents or even grandparents are born small (33, 34).

The high prevalence of morbidities and nutrition problems among LBW infants seem to be associated with poor feeding practices in early life. Exclusive breastfeeding can be challenging in LBW infants due to difficulties in attachment or latching-on, drowsiness, poor and intermittent sucking, poor coordination of sucking and swallowing (35, 36). Problems in breastfeeding lead either to

undernutrition or to use of formula feeding and potentially rapid weight gain, which is also harmful (36, 37). In addition, small size at birth is a stressor to the parents and the families. Mothers of LBW or preterm infants are at increased risk of postpartum depression (38, 39) which in turn can adversely affect childcare during infancy leading to poor health outcomes. There is a need for specialized interventions for LBW infants that can holistically prevent child deaths, illness, and improve nutrition as well as promote positive parental health. But effective interventions that can benefit both the LBW child and its mother, are limited.

For newborns with LBW or preterm birth, putting the babies in incubators for thermal care has become a common practice in many countries and settings, since the 1960s (40, 41). There are several issues associated with use of incubators (42). In less developed countries and settings, availability of modern healthcare technology including incubators is either limited or not used properly due lack of skilled staff or adequate infrastructure. Modern treatment can be quite costly in cases requiring prolonged hospital stay. In low-middle income country (LMIC) settings, incubators are often not used properly, and multiple babies placed in one incubator, leading to frequent nosocomial infections and hypothermia episodes. Most importantly, incubators separate the LBW or preterm babies from their mothers, which impairs mother-infant bonding and delay breastfeeding (42). There is no straightforward solution since the health of an infant is intimately linked to that of its mother and the care she receives during pregnancy, childbirth and postpartum.

2.3 History and Context and of Kangaroo Mother Care (KMC)

"Kangaroo mother care (KMC)" is an unique intervention for improving survival and well-being of LBW infants, defined as both continuous skin-to-skin contact (SSC) of the infant with the chest of the mother (or another caregiver) and feeding exclusively with breast milk (43). The key features of KMC as described by the World Health Organization (WHO) in its 2003 guidelines include early, continuous and prolonged SSC between the mother and the baby; exclusive breastfeeding (ideally); initiated in hospital and can be continued at home (1). KMC is an effective way to meet the needs of the babies born LBW for warmth, breastfeeding, prevention of infections, stimulation, while being close to the

mother (Figure 2). It is to be clarified that KMC is different from routine skin-to-skin contact which is recommended for all newborns during the first hour after birth to ensure warmth and early initiation of breastfeeding (14, 44).

Figure 2. Kangaroo Mother Care

(Photo credits: Society for Applied Studies, Delhi, India)

The concept of KMC was first presented by Dr. Edgar Rey and Dr. Hector Martinez in San Juan de Dios Hospital, Instituto Materno Infantil, Bogota, Colombia in the year 1978 as an alternative against incubator care for LBW or preterm babies. Due to shortage in equipment and staff and high incidence of nosocomial infections, paediatricians at the San Juan de Dios Hospital, sent babies weighing as small as 700 grams to their homes placed vertically in between mother's breasts and fed only mother's milk – these babies were called "kangaroo babies" and the process "kangaroo care"(45). The term 'kangaroo care' was derived due to similarities with marsupial caregiving of the kangaroos, where the vulnerable infant is kept in the maternal pouch to provide warmth and close to the breasts for better breastfeeding.

UNICEF in 1983 reported that "Instead of being placed in an incubator, low birth weight babies are packed close to their mothers right next to the breast. The new technique needs no technology, and its cost is zero…For those weighing between 1000 and 1500 grammes the death rate has dropped from 70% to 10%"(46). Although there were some initial questions raised on the effect of KMC on survival of LBW infants due to lack of clarity on the study population and methodology, currently several well-conducted randomized controlled trials and meta-analysis have established its clear benefit.

2.4 Evidence of Effect of KMC on Clinical Outcomes

Evidence from systematic reviews and meta-analysis have showed that KMC is an effective intervention for preventing deaths in LBW or preterm infants. However, the evidence from the previous individual randomised controlled trials were not very clear maybe because of the small size of the studies.

To investigate the observations of Rey and Martinez on KMC, Charpak and colleagues in 1993 conducted one of the first open-label randomized trial examining the effect of KMC compared to traditional care (incubators) on mortality and morbidity among clinically stable infants with birth weight ≤2000 grams at Clinica San Pedro Claver, Colombia (47). The authors reported a lower proportion of deaths in infants who were randomized to kangaroo care (1.6%, 6/364 infants) compared to the traditional care group (2.9%, 10/345 infants) at 40-41 weeks conceptional age. The study indicated a 41% protective effect against mortality. Follow-up of these infants at 12 months of corrected age showed similar findings with lower deaths in the kangaroo care group (3.1%, 11/339) versus traditional care (5.5%, 19/324), with a 43% protection against mortality (48). However, the 95% confidence interval of the estimates were wide most probably due to small study size.

In the 1990s, several other researchers conducted studies in hospital settings to examine the effect of KMC on child mortality and reported no clear difference in mortality between the KMC and incubator care. Findings from a randomized trial in Ecuador in 1994 that included 300 stabilized LBW babies with birth weight ≤2000 grams showed no substantial differences in deaths among the infants in the KMC group versus standard incubator care group. However, the authors reported a substantially lower rate of serious illness in the KMC group infants (5%, 7/131) compared to the standard incubator care group (18%, 27/152) during a 6-month follow-up period (49). In 1998, a multicentric randomised trial in 3 hospitals in Ethiopia, Indonesia, and Mexico among 285 LBW infants (birthweight 1000-1999 grams), showed no substantial difference in deaths between the KMC group (2%, 3/149) and control group (2.2%, 3/136), but revealed substantial benefits on hypothermia prevention and exclusive breastfeeding at discharge (50).

To collate and summarize the available evidence on KMC, researchers conducted a Cochrane meta-analysis in 2016 (51) including data from 12 randomised controlled trials with 2293 LBW infants. Findings from the meta-analysis showed that the pooled relative risk for death at latest follow-up was 33% lower among infants who practiced KMC against conventional neonatal care (RR 0.67, 95% CI 0.48 to 0.95). The findings also suggested that in clinically stable LBW infants practicing the KMC intervention compared to conventional neonatal care is associated with a substantial 50% reduction in episodes of severe infection or sepsis at latest follow-up, and 72% lesser episodes of hypothermia at hospital discharge or 40-41 weeks postmenstrual age (51).

In the 2016 Cochrane meta-analysis, the authors additionally summarized evidence for effect of KMC on nutritional outcomes. The findings suggested that in LBW infants practicing KMC is associated with substantial 20% improvement in exclusive breastfeeding at 1 to 3 months of age against conventional neonatal care. The authors reported that mean difference (MD) in weight gain at latest follow-up was greater by 4 grams/day in LBW infants practicing KMC against conventional neonatal care. The MD in length gain at latest follow-up was higher by 0.2 cm/week in LBW infants practicing KMC against conventional neonatal care (51).

2.5 Issues Around Initiation and Practice of KMC: Community-Initiated KMC

The World Health Organization in its 2003 guidelines recommended initiation of KMC at the hospital after clinical stabilization of the LBW or preterm infant and to be continued at home after discharge (1). Moreover, initiation of KMC at the community level or at home was not yet recommended by WHO as the evidence on its efficacy and safety was not clearly elucidated (1). LBW infants born at home or those discharged early before KMC initiation, missed-out on the intervention that could potentially save their lives.

In 2008, researchers evaluated the effect of KMC initiated at home or community on neonatal and infant mortality using a cluster randomized trial design in Bangladesh. The trial findings did not suggest any benefits of KMC initiated at home on prevention of child deaths. However, the authors reported several

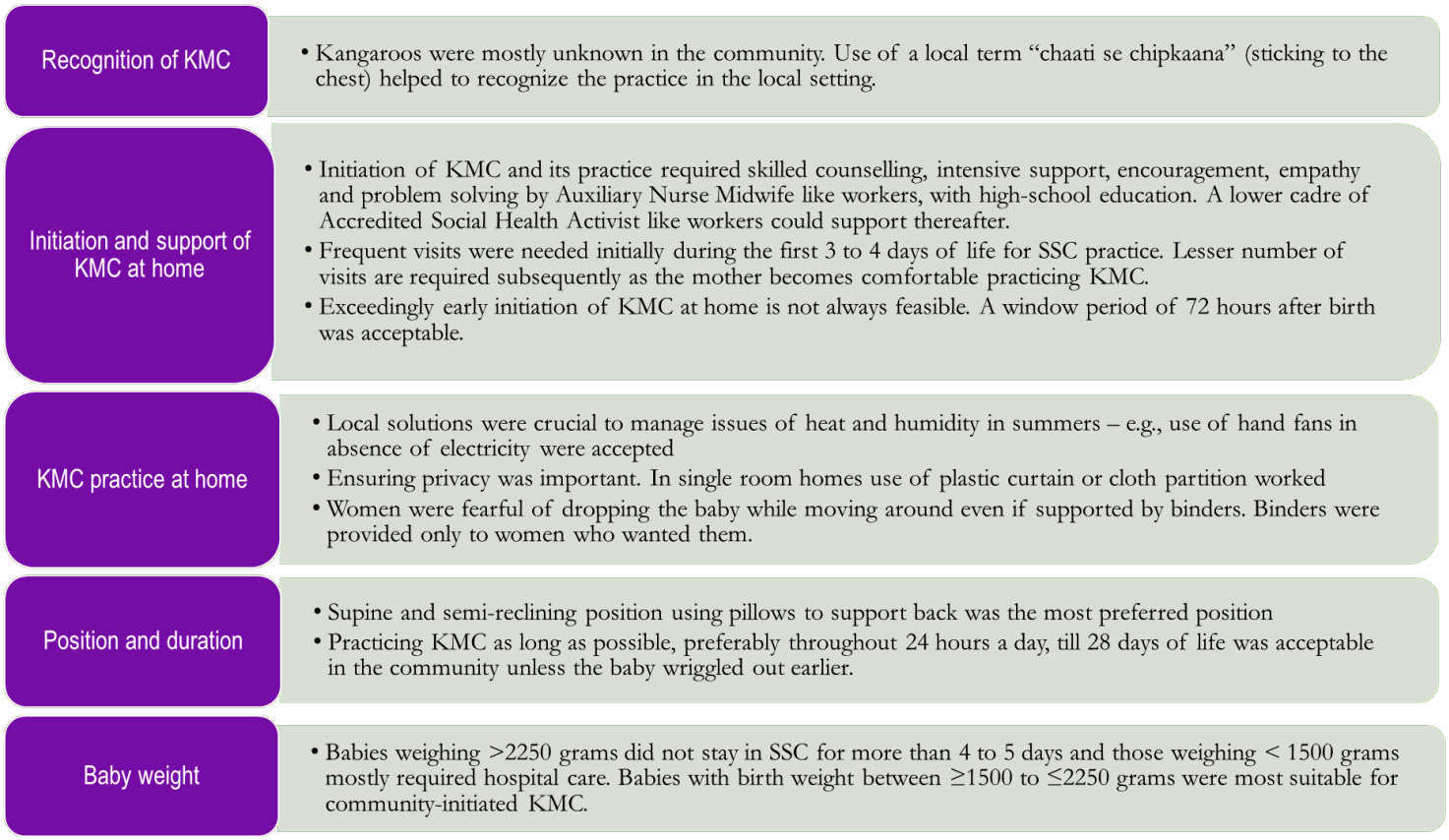
methodological weaknesses of the trial. The authors reported extensive missing records for birth weight (in 40% of the total participants and 65% among those who died within neonatal period) and weak implementation of KMC in the study (in intervention arm mothers, 77% ever practiced SSC and 47% practiced SSC ≥4 hours within the first 2 days of birth). It is possible that the methodological weaknesses may have rendered the study findings unreliable (52).

Despite WHO recommendation, there has been reports of resistance towards implementing KMC in developing countries (11). The Countdown to 2015 report (53) indicated that 33/75 (44%) countries had a policy for KMC practice in LBW babies, yet the coverage of KMC was low or mostly unknown (13, 17). In a multi-country study in 12 Asian and African countries in 2015, authors reported substantial health system related bottlenecks as well as issues in community ownership to support KMC implementation in the country (17).

The identified bottlenecks at the health system level were related to poor leadership and governance, lack of appropriate finances for scale-up, health workforce gaps, inadequate health service delivery systems including issues of space and referral, and poor health information systems. In the community, the major identified bottlenecks were lack of awareness related to KMC and socio-cultural barriers (17). Findings from systematic reviews suggested that lack of confidence in benefits of KMC among health care providers, sub-optimal training, inadequate parental participation, medical concerns like fear of inadvertently hurting the baby were some of the key barriers to KMC practice (54, 55). In addition, the potential benefits of KMC in the mothers are less well understood, which often presents a barrier to its promotion and practice (55).

During 2014-15, investigators from India, conducted formative research to assess the feasibility, acceptability and identify barriers and facilitators of initiating KMC at community-level or home (56). The key lessons (Figure 3) that emerged from the formative research (56) were used subsequently to design the intervention delivery package of a large trial on community-initiated KMC in Haryana, North India (16, 57).

Figure 3. Key Lessons from Formative Research for Initiation of KMC in the Community



In 2019, investigators from India reported findings from a randomized controlled trial conducted during 2015 to 2018, to evaluate the efficacy of promoting community-initiated KMC (ciKMC) on child mortality between enrolment to 28 days age, and from enrolment to 6-month age (16). A total of 8402 stable LBW infants weighing between 1500 to 2250 grams were randomized into ciKMC intervention (n=4480) or control arm (n=3922). Between enrolment to 28 days of life, the study reported 73 infant deaths in 4423 periods of 28 days in the intervention arm and 90 deaths in 3859 periods of 28 days in the control arm. The findings suggested a substantial 30% improvement in neonatal survival as an effect of KMC promotion. Between enrolment and 180 days of life, there were 158 infant deaths in 3965 periods of 180 days in the intervention arm, and 184 infant deaths in 3514 periods of 180 days in the control arm. The findings suggested a substantial 25% improvement in birth to 6-month survival as an effect of KMC promotion. The trial also reported substantial clinical benefits on prevention of possible serious bacterial infection in the neonatal period, prevention of diarrhoea with dehydration and pneumonia. In the intervention arm against control arm, the study reported a lower proportion of infants with severe underweight (15% lower), severe stunting (7% lower), and severe wasted (27% lower) at 28 days of age (16). Severe underweight was defined by weight for age Z score <-3, severe stunting was defined as length for weight Z score <-3, and severe wasting was defined as weight for height Z score <-3 as per WHO 2006 growth standards (58).

2.6 Pathways of Effect of KMC: Summary of Evidence and Knowledge Gaps

Research have demonstrated that KMC either initiated at hospital or home has large clinical benefits in LBW infants on health and survival. However, understanding on the pathways or intermediate biological outcomes through which KMC works to prevent mortality and morbidity is sketchy. Among many health care providers, there is a perception that the clinical benefits of KMC are possibly due to closer attention to the child and hypothermia prevention with limited value where incubators are available. The full range of benefits of KMC is unclear to the heath care workers and the caregivers (55).

It is suggested that the clinical benefits of KMC on infant health and survival may operate through a multitude of pathways or intermediate biological outcomes (59). Some of the key plausible biological outcomes through which KMC works to improve infant health and survival are 1) hypothermia prevention, 2) stabilization of vital physiological parameters, 3) enhanced infant nutrition (weight and length) by better breast milk output and improved infant breastfeeding performance, 4) improved infant gut function i.e., reduction in infant gut inflammation and permeability, 5) establishment of a healthy gut microbiota rich in Bifidobacteria and less of harmful Gammaproteobacteria, through successful breastfeeding and reduced exposure to pathogenic bacteria, and 6) reduction of maternal postpartum depressive symptoms and stress through better mother-infant bonding and therefore better care of the baby.

2.6.1 Hypothermia Prevention

Hypothermia is defined as body temperature less than 36.5ºC. LBW or preterm infants are very vulnerable to hypothermia because of low subcutaneous fat and relatively large body surface area with respect to weight. Hypothermia is a major cause of neonatal illness and death in LBW infants (42). Evidence from randomised controlled trials have shown that KMC practice can substantially prevent hypothermia episodes in LBW infants, thereby preventing deaths (51, 60). In a meta-analysis in 2016 that included 9 hospital-based randomised trials, authors reported a 72% lower risk in the episodes of hypothermia at discharge among LBW infants in the KMC group compared to conventional neonatal care (51).

2.6.2 Stabilization of Vital Physiological Parameters

Several circulatory, cardiac, and respiratory adaptations are needed at birth for the lungs to successfully replace the placenta as the site of gaseous exchange (61). Infants born low birth weight or premature often have an immature cardiorespiratory system requiring life-saving medical support. These LBW or preterm infants are therefore often placed in incubators in intensive care units to provide cardiorespiratory support.

Evidence from hospital-based studies indicate that practice of KMC is effective in stabilization of infant vital physiological parameters including heart rate, respiratory rate, oxygen saturation and blood glucose in comparison with incubator care (23, 62-64). Findings from previous reviews suggest that in infants practicing KMC compared to incubator care, heart rate and respiratory rate were not substantially different and within clinically acceptable range (63, 65). Events of bradycardia or tachycardia and episodes of oxygen desaturation or apnea have been observed to be lower in infants practicing KMC compared to incubator care (62, 65). Reports from randomized trials show that blood glucose concentration within 2 hours of birth is higher in newborns practicing KMC as against incubator care, suggesting a lower risk of hypoglycemia (23). The available evidence supports the hypothesis that KMC practice can prevent LBW newborn deaths through stabilization of vital cardiorespiratory parameters.

2.6.3 Infant Breastfeeding Performance

Breast milk is the primary source of nutrition in young infants. The WHO recommends exclusive breastfeeding (EBF) in the first 6 months of life for all infants including LBW (66, 67). Exclusive breastfeeding practice can promote growth and survival in LBW infants, confer protection against infections, and prevent childhood undernutrition (37). In LBW infants, prevalence of EBF practice is lower compared to infants with birth weight ≥2500 g (68). In a multicountry cohort, the practice of non-EBF at six weeks after birth in LBW infants against infants with birthweight ≥2500 g was found to be 30% higher in India and 20% higher in Guatemala (68). Challenges in EBF practice in the infants born LBW were difficulties in latching, poor sucking, drowsiness, poor coordination between sucking and swallowing (35, 69). Poor infant breastfeeding behaviour is also associated with maternal stress and perceived breastfeeding insufficiency (35, 70).

In 2016, researchers conducted a meta-analysis including 5 hospital-based randomized trials that indicated a substantial 1.2 times higher exclusive breastfeeding rates among LBW infants in the KMC group against those in the control group at 1 to 3 months of age (51). I did not find existing literature on the effect of KMC practice on breastfeeding behaviour including effective latching and sucking in the LBW or preterm infant. In mammalian biology, SSC

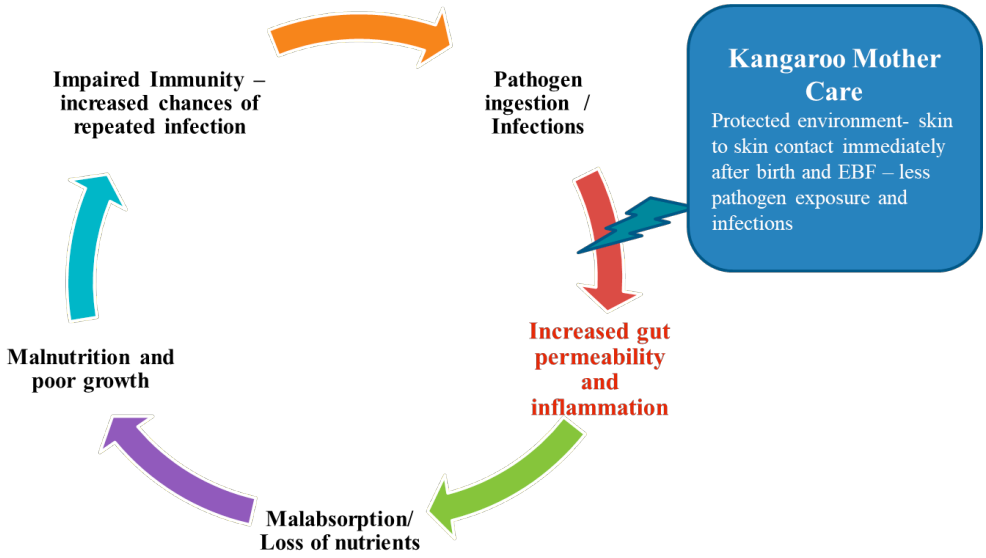
can promote self-attachment of the infant to the mother's breast, resulting to early breastfeeding initiation (19, 71-74). The SSC component of KMC, through sensory stimuli such as touch, warmth and odour, acts as a powerful vagal stimulant and facilitate release of maternal oxytocin (18). The oxytocin hormone is associated with reduction of maternal anxiety, increased maternal confidence, improved attachment, better milk output, and successful breastfeeding (75, 76). Hence, it is plausible that KMC promotion can improve infant breastfeeding performance in LBW infants.

2.6.4 Infant Gut Function

Gut function among infants residing in low-middle income communities is suggested as an important driver of poor growth. Environmental enteric dysfunction (EED) is a broad syndrome seen commonly in low-middle income settings, presenting with several alterations in gut function including, increased gut inflammation, altered gut permeability, crypt hyperplasia and villous blunting (77). EED can induce a vicious cycle of reduced intestinal absorption, which causes protein-energy and micronutrient deficiencies, and lead to poor growth (Figure 4) (77-79). Currently, there is no universally accepted case definition for EED (80), however several fecal biomarkers have been identified to measure components of EED like gut inflammation and permeability (81).

Prevention of EED is important in infants born LBW who are already at increased risk of enteric infections and growth faltering. Studies have tested interventions like antibiotics or probiotics, improved infant feeding, zinc supplementation, or water, sanitation, and hygiene (WASH) but have failed to demonstrate a clear effect in improving gut function, inflammation, and permeability (82-85). I did not find existing literature on the effect of KMC practice on gut function including gut inflammation, or permeability in LBW or preterm infants. It is possible that in KMC as the LBW infant is placed in a safe and protective environment in SSC with the mother soon after birth and is exclusively breastfed, the likelihood of repeated pathogen exposure and ingestion of microbes from contaminated sources are less (21, 86). Therefore, it is conceivable that KMC might lead to a reduction in gut inflammation and permeability.

Figure 4. Cycle of Impaired Gut Function and Poor Nutrition



2.6.5 Infant Gut Microbiome

Alterations in gut microbiota has been demonstrated to be associated with infant undernutrition (87, 88). Transfer of microbiota from undernourished children to germ-free mice, was found to impair the physical growth (weight) in the mice, and that from well-nourished children prevented growth impairment (89). In the newborn period, the intestinal microbiota is initially colonized by Enterobacteria. In exclusively breastfed infants, the microbiome changes by around 1 month of age and comprise predominantly of Bifidobacterium group which are milk oligosaccharide fermenters (88, 90). Infant gut microbiome with abundance of Bifidobacterium is reported to be associated with modulation of infant host responses and infection prevention (88). In preterm or LBW infants gut microbiome is predominantly composed of Gammaproteobacteria group with Klebsiella, Enterococcus, Escherichia and Shigella as the most common genera, with reduced abundance of Bifidobacterium (91).

Previous evidence show KMC can improve child nutrition outcomes including prevention of infant wasting, and possibly improving weight gain and length gain (16, 92). It is possible that the effect of KMC on enhancing infant nutrition may be mediated through establishment of a Bifidobacterium rich gut

microbiome among infants born LBW through maternal transfer, better breastfeeding performance, exclusive breastfeeding, and reduced exposure to harmful bacteria e.g., Klebsiella, Escherichia, Shigella.

2.6.6 Maternal Postpartum Depressive Symptoms

Infant health is closely linked to its mother and her well-being. Postpartum depression (PPD) in mothers affects quality of life and is associated with several negative effects on infant health. PPD leads to poor mother-child interaction, as well as poor breastfeeding which can affect child growth and development (93-95). It is estimated that around one-fifth of the mothers in low- and middle-income countries have postpartum depression (96). The risk of depression during 12 weeks after birth seems to be higher among mothers who give birth to small preterm infants compared to healthy term infants (38, 97).

It is conceivable that KMC can reduce the risk of postpartum depressive symptoms through better mother-infant bonding by stimulating the sensory pathways and possibly via facilitating enhanced maternal oxytocin release and reducing cortisol secretion (18, 22, 23). Data from observational and non-randomised studies indicate such a beneficial effect on mothers (98-101). In 2009, findings from an observational study in Portugal, among 177 mothers with preterm birth, showed that the proportion of women with postpartum depression assessed using the Postpartum Depression Screening Scale declined from 37% after birth to 17% at hospital discharge with KMC practice (99). In 2010, researchers from Iran conducted a quasi-experimental study to evaluate the effect of KMC practice in LBW infants on maternal mental health scores assessed by the General Health Questionnaire among 50 mothers. The authors reported that 180-minute daily practice of KMC for a week compared to incubator care was associated with a higher mean maternal mental health scores in the KMC group (101). But there is a need for better quality evidence from randomized trials.

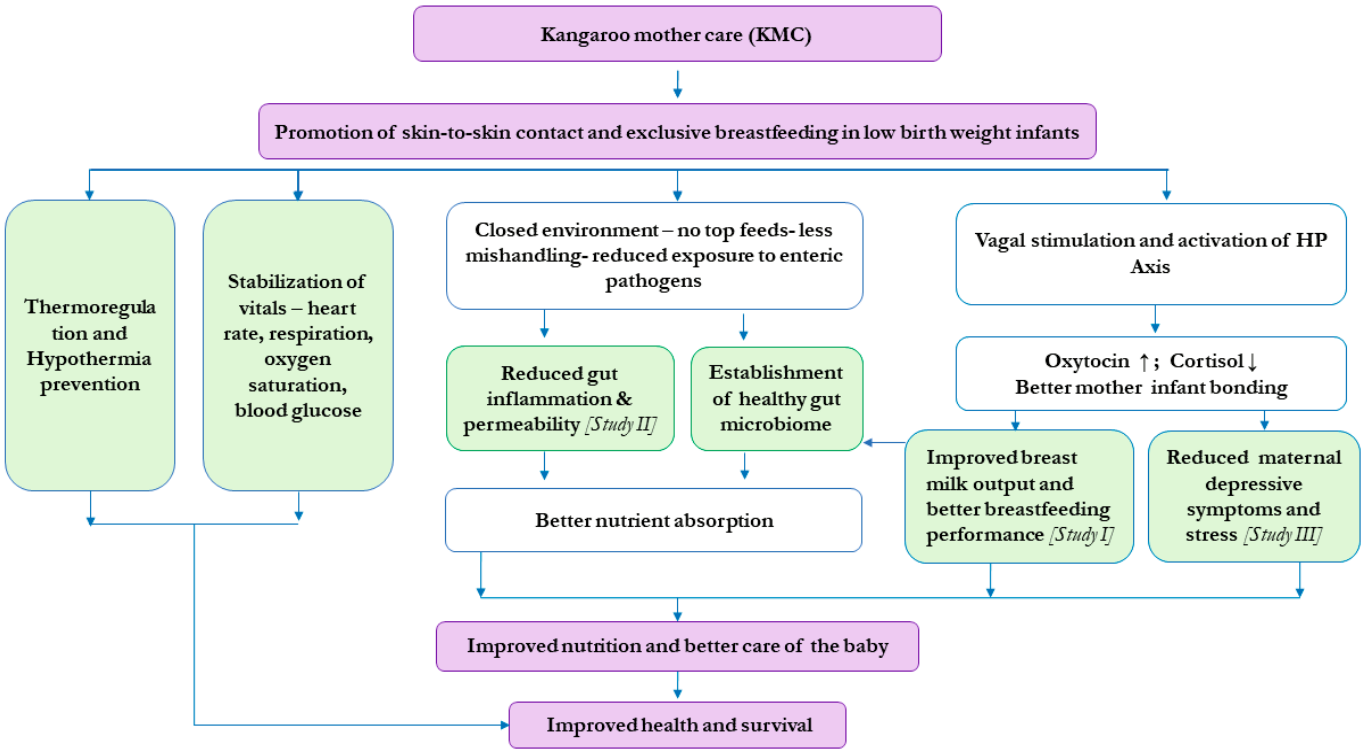
2.7 Theoretical Framework and Justification of the PhD Thesis

Available literature suggests that the clinical benefits of KMC on health and survival of LBW infants might operate through several pathways involving different biological outcomes including hypothermia prevention, stabilization of vital physiological parameters, infant breastfeeding performance, infant gut

function, infant gut microbiome, and maternal postpartum depressive symptoms. Previous research has demonstrated that KMC practice can prevent hypothermia and help in stabilization of vital physiological parameters. However, the effect of KMC on infant breastfeeding performance, infant gut function, infant gut microbiome, and maternal postpartum depressive symptoms is yet to be clearly elucidated.

The studies included in the PhD thesis were planned to evaluate the effect of KMC on three key biological health outcomes (I) infant breastfeeding performance, (II) infant gut function, and (III) maternal postpartum depressive symptoms (Figure 5). My hypothesis was that KMC can improve infant breastfeeding performance and reduce the risk of maternal postpartum depressive symptoms through improved mother-infant bonding and improve infant gut function through reduced pathogen exposure and various anti-infective, anti-inflammatory substances in breast milk (102).

Figure 5. Conceptual Framework for Plausible Pathways of Effect of KMC to Improve Health and Survival in Low Birth Weight Infants



3 AIMS

The current study in low-middle income settings in India was set to evaluate the effect of promotion and support of kangaroo mother care (KMC) in stable low birthweight (LBW) infants on some important maternal and child health biological outcomes.

The specific aims were to:

1. Estimate the effect of promotion and support of KMC among stable LBW infants in low-middle income populations in India or similar settings in South Asia, on effective breastfeeding performance at the end of the neonatal period.

2. Estimate the effect of promotion and support of KMC among stable LBW infants in low-middle income populations in India or similar settings in South Asia, on fecal biomarkers of infant gut function at the end of the neonatal period.

3. Estimate the effect of promotion and support of KMC promotion among stable LBW infants in low-middle income populations in India or similar settings in South Asia, on the risk of maternal postpartum depressive symptoms at 28 days after birth.

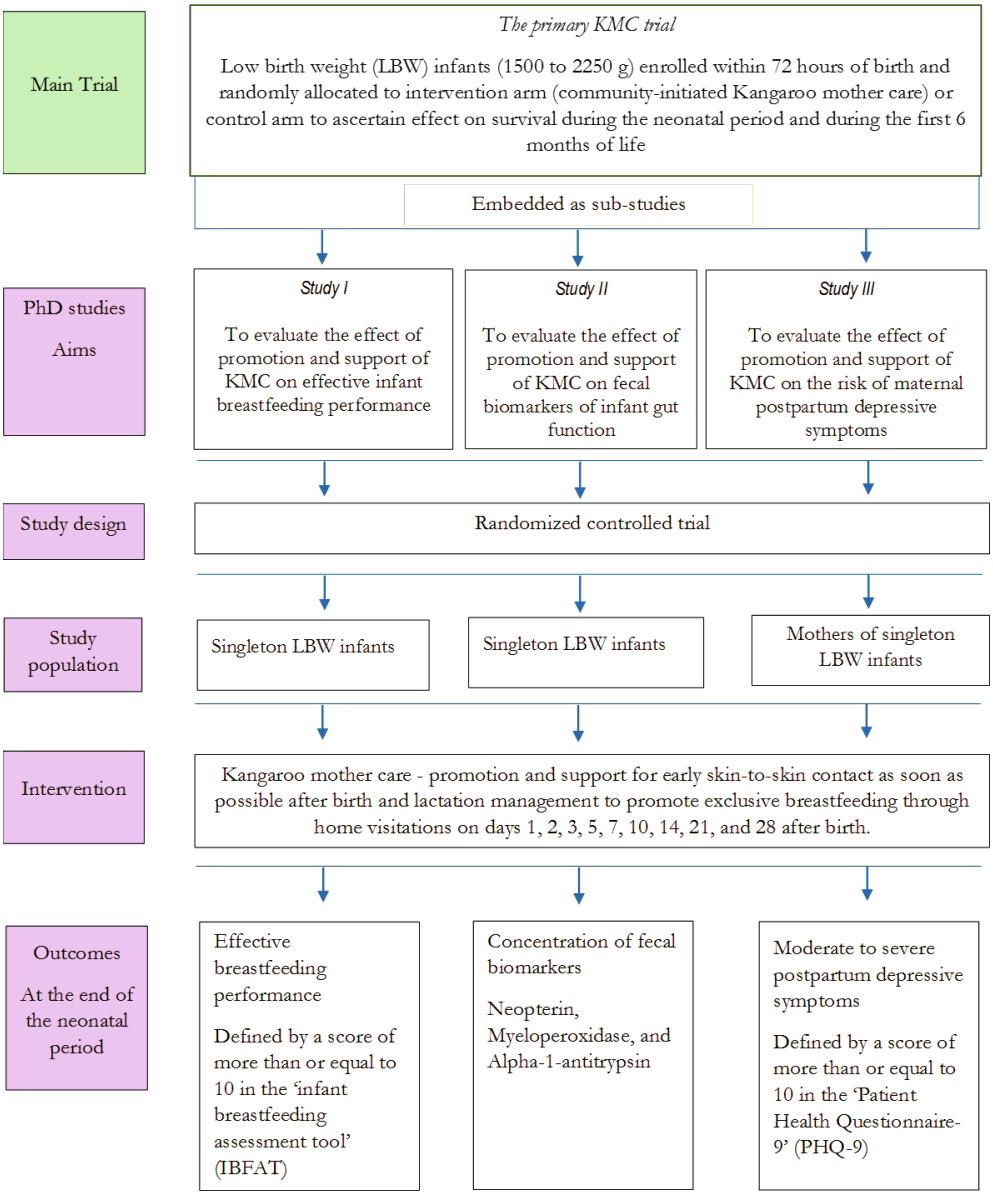
4 METHODS

4.1 Approach to the Study

To address the study aims, three sub-studies were designed as randomized controlled trials embedded within the larger primary KMC trial titled "Impact of Community-initiated Kangaroo Mother Care on Survival of Low Birth Weight Infants" (www.clinicaltrials.gov #NCT02653534)(16) in Haryana, India, to study efficacy of the intervention (Figure 6). These three studies (referred to as Study I, II and III) constitute the PhD thesis.

Eligible participants from those included in the primary KMC trial were approached consecutively (Study I and II) or selected randomly (Study III) for obtaining additional consent for assessment of the study outcomes until the sample size for each study was attained. The randomization sequence of the primary KMC trial was used in all the three studies. Outcome assessment for three studies were conducted by separate outcome assessment teams that had neither been involved in delivering the KMC intervention nor in collecting data on outcomes for the primary trial. The details of the design, including enrolment and randomization, intervention, and outcome assessment are described below in separate sections.

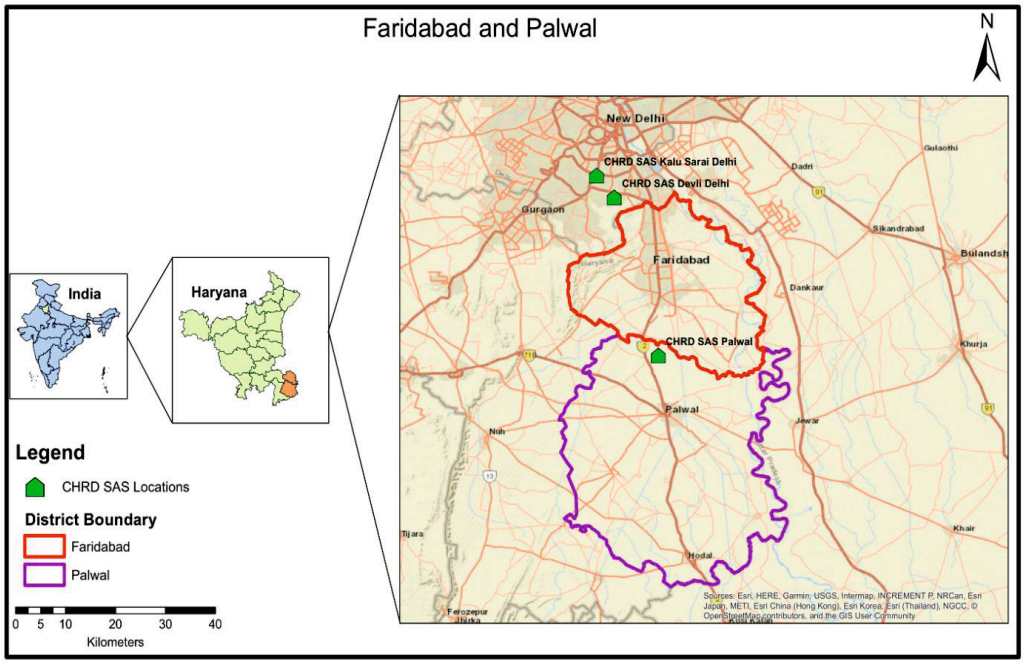
Figure 6. Overall Design of the PhD Studies



4.2 Study Settings

The studies were set in rural areas of the districts of Faridabad and Palwal in the Indian state of Haryana (Figure 7). The study areas were selected due to their high prevalence of low birth weight and home births. For the last 20 years, investigators of Centre for Health Research and Development, Society for Applied Studies (CHRD, SAS) have conducted several maternal and child health projects in this area. The researchers of the Institute have developed an excellent rapport with its population and administration. The field office of the Institute was in the Tatarpur area of the Palwal district, Haryana (Figure 7).

Figure 7. Map of the Study Area



*Prepared using ArcGIS software version 10.8

The overall population in the study area was around 2 million when the studies started (103). In this setting, less than half of the families were nuclear; the median number of family members was six. The area comprised of low- to-middle income socioeconomic neighbourhoods with annual household income between 2500 to 3500 USD. The sex ratio in the area was 926 females per 1000

males (104). The common occupations for males were work in factories or commercial enterprises (38%), daily wage construction workers (20%), self-employed as shopkeepers (22%), government services (5%); 10% farming, and around 5% unemployed. Among women, 95% did not work outside home (103). The median (range) years of schooling for men was 9 years (5 to 11) and 3 years (0 to 8) for females; around 40-50% of women had never been to school (15, 103). Common sources of drinking water were hand pumps (35-40%), piped water (28-30%) and public taps (15-20%). Around 50% of the population use open fields for defecation. A high proportion of families (92%) have electricity in their homes (103).

During 2014-16, the annual birth rate in the area was 25 per 1000 population. Around 60% of the births in the area were in institutions (15, 103). Around one-fourth of the babies were born with low birth weight (103). The most common sources for seeking care for children with illness were private practitioners within and outside the villages (~60%) (103).

The government of Haryana provided primary health care in the study area through the Primary Health Centers (PHC). Each PHC catered to a population of approximately 30,000. Under each PHC, there were 4 to 6 subcenters, each of which catered to around 5000 people. Each subcenter was run by a female health worker known as the auxiliary nurse midwife (ANM). Each ANM was supported by 4-5 Accredited Social Health Activists (ASHA), who represented the first level of contact between the community and the health system. At the time of study initiation, there were a total of 25 PHCs in the area; 12 in Faridabad and 13 in Palwal district.

Each ASHA catered to approximately 1000 people in the community (105). She was responsible for providing home based newborn care (HBNC). HBNC program is the standard newborn care provided at home through the government health system throughout the country, including the study area, for all newborns including those born LBW or preterm (106). The ASHAs role was to record (a) the weight of the newborn in the Mother Child Protection card, (b) ensure complete immunization till 6 weeks of life (c) counselling on breastfeeding, postpartum care, family planning, identification and referral of any illness towards ensuring safety of both the mother and the newborn till 42 days after birth, and (d) completion of birth registration (106). At study start in 2014,

as per the home-based newborn care (HBNC) policy, ASHAs were to visit the homes of the newborn on days 3, 7, 14, 21, 28 and 42 of life and additionally on day 1 for babies born at home. In addition, as per the HBNC program, the ASHAs were to visit the infants born LBW or preterm further every quarter from 3 months till 1 year of age, i.e., at 3, 6, 9 and 12 months. The 2014 HBNC program guidelines has no mention of promotion of kangaroo mother care or skin to skin contact at home in LBW or preterm infants may be because its efficacy was not yet established (106).

4.3 Study Participants: Inclusion, Exclusion, and Enrolment

The study participants were mothers and their LBW infants enrolled in the primary KMC trial. In the primary KMC trial, all live births in the area were tracked by a community-based pregnancy surveillance. The surveillance population included all women of reproductive age (15-49 years) living permanently in the area. Pregnant women were identified by a door-to-door survey conducted every 3 monthly by a pregnancy, screening, enrolment (PSE) team. The PSE workers contacted the women telephonically or, if phone calls were unsuccessful, by home visits. Contacts were made monthly in the first or second trimester, twice weekly in the third trimester, and daily closer to delivery. Identified pregnant women were recorded in a surveillance register and were followed up until delivery or other pregnancy outcome. If a baby was born in a hospital, the PSE team worker followed up the mother and baby until they were discharged.

Babies were weighed and screened at home by the PSE worker based on study inclusion and exclusion criteria after written informed consent was obtained from the mother or caregiver. The process of consenting and screening for enrolment was conducted as early as possible, preferably within 12 hours and not later than 72 hours of birth.

In the primary KMC trial, we included mothers and their infants between July 2015 to October 2018 if they weighed between ≥1500 to ≤2250 grams within 72 hours of birth, born at home or at hospital with KMC not initiated in hospitals. The birth weight was measured using AWS-SR-20 (American Weigh Scales, Cumming, GA, USA; sensitivity 10 grams) at home by our trained study team

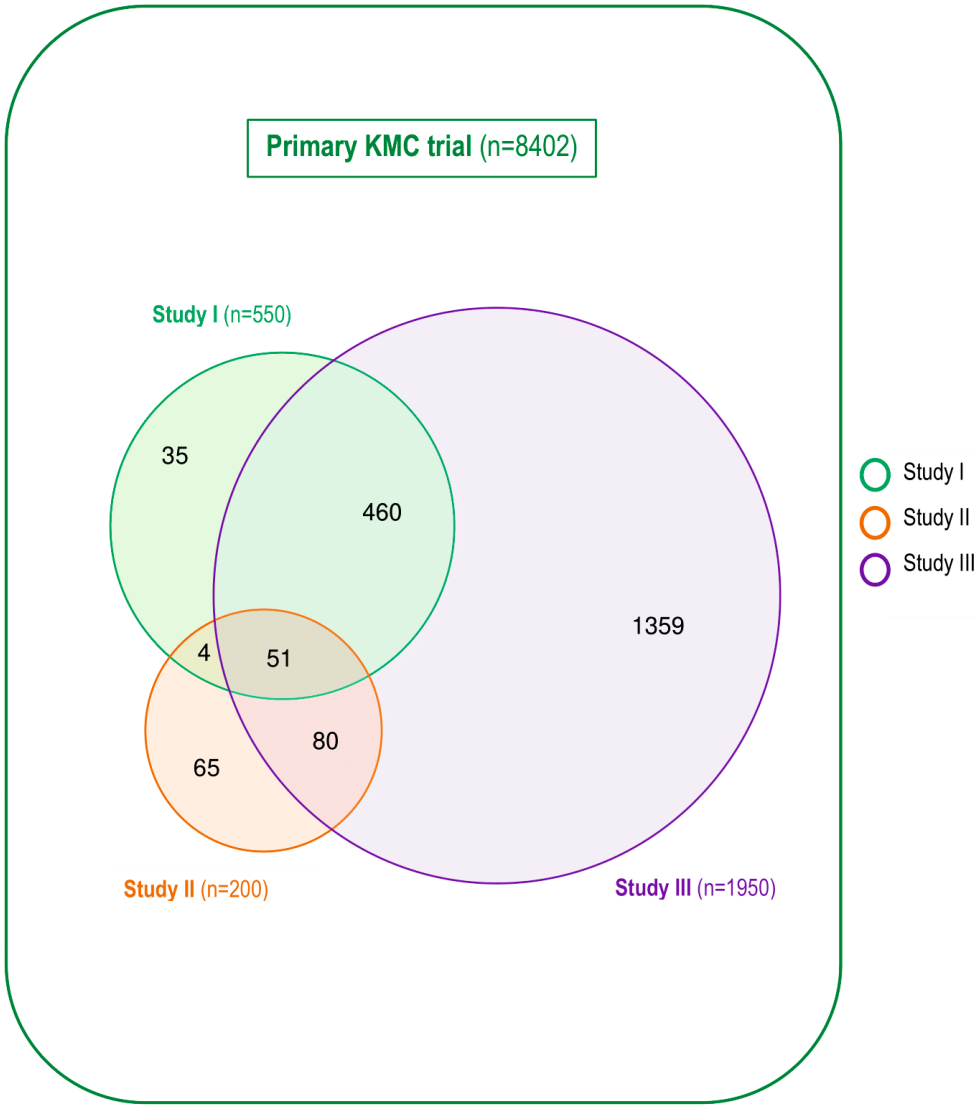
workers within 72 hours of birth. We did not include infants weighing <1500 grams as our formative research showed that these infants can be unstable, have respiratory or feeding problems, and often need prolonged hospitalization. As newborns weighing >2250 grams tend to wriggle-out of the KMC position early i.e., considerably earlier than the end of the neonatal period, we included infants weighing up to 2250 grams (56). LBW infants were eligible to be included in the trial if KMC was not initiated in the hospital. Infants unable to feed, with breathing problems, major congenital malformations or less active than normal (as per mother's report) on the day of visit were not included and were referred to nearby hospitals. Those intending to move away over the next 6 months were also excluded.

For the studies included in the PhD thesis, only singleton LBW babies were enrolled. To obtain consent, the worker described the purpose of the study, its potential risks, and indirect benefits of participation. No monetary benefits were offered. The mother was informed about the confidentiality of the information collected and that her name and name of the infant would not be used or disclosed anywhere. The study worker read out the information sheet in Hindi, or the mother read it herself, if she so preferred. For participants who were unable to read, the consent form was read out by the worker in the presence of an impartial literate witness. For participants who could not sign, a thumb impression was taken which was witnessed and countersigned by the witness. Consenting and enrollment in the studies were done by Day 7 of birth. Further, in Study I (Infant breastfeeding performance)(107) children who were not yet being breastfed were excluded. In Study II (Infant gut function) (108) we did not include more than one infant from each household because of common water, sanitation of hygiene practices. In Study III (Maternal postpartum depressive symptoms) (109) only mothers of singleton infants were enrolled.

For Study I, 550 eligible infants and their mothers were enrolled consecutively from April 2017 onwards. For Study II, consecutive 200 eligible infants from May 2017 onwards were enrolled. For Study III, an a priori decision was made for feasibility reasons, to restrict the number of enrollments to a maximum of 6 per day. Between April 2017 and March 2018, 1950 of the 3326 eligible mothers enrolled in the primary KMC trial were included in this study. Study I and III were initiated together, while Study II was initiated a month later. Among the participants included, 51 participants were enrolled in all three studies, four

participants were part of Study I and II, 80 of Study II and III, and 460 of Study I and III (Figure 8). Therefore, overlap among the study participants was lowest between Study I and II.

Figure 8. Overlap Between the Participants in the Studies Included in the PhD Thesis



*The numbers in the Venn diagram figure indicate the number of study participants. Study I: Infant breastfeeding performance; Study II: Infant gut function; Study III: Maternal postpartum depressive symptoms

4.4 Randomization

The randomization sequence of the primary KMC trial (16) was used in the studies included in the PhD thesis. In the primary KMC trial, eligible LBW infants were randomly allocated in a 1:1 ratio into the intervention or control arm. The randomization list, with permuted blocks of variable sizes i.e., 4, 6 or 8, was prepared by an off-site statistician at the WHO Headquarters in Geneva who was not otherwise involved in the study. For allocation concealment, sequentially numbered, opaque sealed envelopes were used. The envelopes were kept under lock and key in a cabinet until use, accessible only to the randomisation coordinator. In eligible infants, after consent was obtained, the PSE team worker contacted the randomization coordinator. The randomization coordinator opened the envelope and sequentially assigned a unique participant identification number. If the child was allocated to the intervention arm, the unique participant identification number of the infant was communicated to the enrollment team worker and to the intervention delivery team (16).

It was not possible to mask the participant mothers or the study teams because KMC was a behavioural intervention. However, we deployed separate teams for intervention delivery and outcome assessment.

4.5 Intervention

The intervention included promotion and support for early skin-to-skin contact (SSC) as soon as possible after birth and lactation management to promote exclusive breastfeeding (16). The intervention was delivered by a team of trained intervention delivery workers and their supervisors. The workers had 10-12 years of schooling which is similar to the educational background of the ASHAs. They were selected from the study area to ensure that they were familiar with the socio-cultural environment and had access to the households. The supervisors were college graduates, with educational qualifications similar to that of the government ANMs.

The intervention delivery team was intensively trained in supporting skin-to-skin contact, lactation management and counselling, by trainers from Swami Dayanand Hospital, New Delhi, a tertiary care hospital. This included sessions

on KMC practice in hospital wards. Thereafter, the workers had hands-on training in the field area to initiate and support KMC.

The intervention delivery team visited homes of the children allocated to the intervention arm as early as possible to initiate KMC. The team used photographs and a local term "chaati se chipkaana", which means sticking the baby to the chest, to explain the essential features of KMC. The team counselled the mother and other family members to practice SSC as long as possible during the day and night, with the assistance of the father and other family members. The mother was taught correct positioning and attachment during breast feeding and how to keep the baby in the SSC position. Other family members were encouraged to practice SSC particularly in case the mother was unable to provide SSC because of illness, pain after delivery, or other reasons. If the baby was too weak and unable to suck effectively, the mother was taught how to express breast milk, which was then fed to the child with a cup and spoon until she or he could suckle.

The team scheduled visits to the homes of the families on days i.e., 1, 2, 3, 5, 7, 10, 14, 21, and 28 after birth. During these home visits, the team observed the SSC practice and breastfeeding, and helped to resolve any difficulties related to KMC practice. The workers of the intervention delivery team recorded information on duration of SSC practice daily during their scheduled visits. The duration of each home visit ranged from 30 to 45 minutes. During the visits, in case the mother or family members asked about growth of the baby, the intervention team worker weighed the baby and informed the family. In case the child needed referral due to any illness, the study intervention team worker facilitated referral through the government ASHAs to the nearest health facility. The visits continued until the infant wriggled-out from the KMC position and no longer accepted SSC or until 28 days of age, whichever was earlier.

The intervention team was supported by a process evaluation team, which comprised of experienced study coordinators who accompanied the intervention delivery team members. During these visits, the coordinators observed how the intervention workers counselled and supported the mother for KMC and their ability to resolve problems. They also observed the interaction of family members with the mother, family support to the mother, physical ambience and home environment, mothers' condition, and caring practices for the baby. Each

intervention delivery team member was observed at least once every 3 months. Weekly meetings were conducted to share and discuss the experiences, identify barriers and enabling factors, solve problems, and retrain the intervention delivery workers. All infants in the intervention and control arms of the trial received standard of care i.e., home-based newborn care (HBNC) visits by ASHAs as implemented through the government health system (106). As per the HBNC program, ASHAs were to visit the homes of the newborn on days 3, 7, 14, 21, 28 and 42 of life and additionally on day 1 for babies born at home.

4.6 Outcomes and their Assessment

In this section, I have described the outcomes in order of the study objectives. Outcome assessment was similar across all participants in the intervention or control arm. The definition or description of the outcomes are mentioned first, followed by methods of assessment.

4.6.1 Study I: Infant Breastfeeding Performance

In Study I, the primary outcome was 'effective breastfeeding performance' at the end of neonatal period (i.e., 28 days after birth). Effective breastfeeding performance was defined by a score of more than or equal to 10 in the 'infant breastfeeding assessment tool' (IBFAT) (Appendix 1) (110). The median IBFAT score with its interquartile range (IQR) was also reported.

In addition, the proportion of infants showing 'effective readiness to feed', 'effective rooting', 'effective fixing', and 'effective sucking pattern' were reported as secondary outcomes. Each of these are components of the IBFAT and are scored from 0 to 3 on a Likert scale based on observed feeding. A score of '0' indicated poorest performance and '3' indicated effective performance. The total IBFAT score accordingly ranged from 0 to 12 (110).

The IBFAT is a reliable instrument that was previously validated and used for assessment of breastfeeding performance in infants (20, 111). Previous studies have reported high correlation ($R > 0.7$) between IBFAT and other available

tools such as 'LATCH' and the 'Mother Baby Assessment' tool for assessment of infant breastfeeding performance (112).

The other secondary outcomes were 'maternal satisfaction related to infant breastfeeding' (7-day recall) and 'exclusive breastfeeding' (24-hour recall) at the end of the neonatal period. 'Maternal satisfaction related to infant breastfeeding' was assessed using a 4-point Likert scale where the mother was asked if she was 'very satisfied', 'satisfied', 'somewhat satisfied' or 'not satisfied' with the way the baby breastfed during the last 7 days. The scale was originally developed in 1988 (110) and thereafter adapted and used in different settings, including in India (110, 111).

Assessment of the breastfeeding performance outcome in all study participants was conducted at home by an independent study outcome assessment team of four trained female workers who had graduate-level education. Assessment was conducted at enrolment and at the end of the neonatal period. This team was not involved in intervention delivery or ascertainment of the duration of SSC which was done by the primary KMC trial outcome assessment workers. The team was trained by an expert in infant and young feeding counseling certified by the 'Breastfeeding Promotion Network of India' (https://www.bpni.org). After the initial training sessions, a standardization exercise was conducted, in which the IBFAT scores assigned by the outcome assessment team workers were compared with that of the certified expert for agreement. The workers were eligible to conduct study assessments if the score obtained in all the four components of IBFAT had a interclass correlation coefficient of >0.75 (113) compared to the expert.

4.6.2 Study II: Infant Gut Function

In Study II the primary outcomes were the concentration of the fecal biomarkers neopterin (nmol/L), myeloperoxidase (ng/mL), and alpha-1-antitrypsin (µg/mL), at the end of the neonatal period. Neopterin is a marker of T-helper cell 1 activity and indicates gut inflammation. Myeloperoxidase is another indicator of gut inflammation that reflects neutrophil activity in the intestinal mucosa. Alpha-1-antitrypsin is an indicator of intestinal permeability and protein loss. Enteric enteropathy (EE) score and EE index (81, 108) were estimated

using the concentrations of these three biomarkers and were reported as secondary outcomes.

For all study participants, stool specimens were collected at baseline within seven days of birth, and within one week after the end of the neonatal period. A stool kit containing a gamma-irradiated stool container in a cold box was provided to the mother. The study worker informed the process of fecal specimen collection to the mother and family members. The mother was advised to collect at least 5g of the baby's stool (in the marked container) as soon as after stool passage, store it in the cold box, and inform the study worker immediately. The study worker collected the cold box with the specimen and transported it to the Clinical and Research Laboratories, Society for Applied Studies (CRL SAS), New Delhi within 6 to 8 hours. To ensure proper transport of specimens to the laboratory, a standard checklist of activities was followed, a specimen transport register, and a lab receiving register was maintained. For operational feasibility to enable prompt transportation to the laboratory, the mother was asked to collect baby's stool between 6 AM and 3 PM. The specimens were stored in a -80^{0}C freezer until analysis without any fixatives.

Fecal biomarkers of gut function were analyzed in CRL SAS, New Delhi using the automated 'EVOLISTM Twin Plus' ELISA system (BioRad, California, USA). For neopterin assessment, the 'IBL International Kit' (Hamburg, Germany) was used. For myeloperoxidase, the 'K6630 IDK MPO ELISA kit' (Immundiagnostik AG, Bensheim, Germany) was used. The 'Human A1AT kit' (Immuchrom Gmbh, Heppenheim, Germany) was used for alpha-1-antitrypsin assessment. Before conducting the analyses, the three kits were verified in the laboratory settings to identify the acceptable range of values for accuracy, inter- and intra-assay precision, and linearity, using the manufacturer standards and instructions. Specimens with values out of acceptable range were diluted as required and the dilution factor was accounted for.

4.6.3 Study III: Maternal Postpartum Depressive Symptoms

In Study III the primary outcome was 'moderate-to-severe postpartum depressive symptoms' in mothers at 28 days after birth. History of postpartum depressive symptoms in the past 14 days was assessed using a pre-tested Hindi

version of the validated 'Patient Health Questionnaire-9' (PHQ-9) (Appendix 2) (114, 115). Moderate to severe postpartum depressive symptoms was defined by a PHQ-9 score of ≥10. The sensitivity of a PHQ-9 score ≥10 to diagnose major depression in the postpartum period is reported to be 80%, and the specificity 90% (115, 116).

Any postpartum depressive symptoms (PHQ-9 score of ≥5), PHQ-9 score values (on a continuous scale), and maternal salivary cortisol concentration at the end of the neonatal period were reported as secondary outcomes. PHQ-9 scores were categorized as follows – 0 to 4 was 'no or minimal', 5 to 9 was 'mild' and ≥10 was 'moderate-to-severe' postpartum depressive symptoms (114).

Assessment of postpartum depressive symptoms was conducted using PHQ-9 at home for all study participants by an independent study outcome assessment team of six trained female workers who had graduate-level education. The workers of the team were trained and assessed by a clinical psychologist. The team was trained to conduct the PHQ-9 assessment based on an interview process and not asking the questions directly. The PHQ-9 assessment was done between day 28 up to day 42 of birth. Random quality checks were done in 1% of the assessments by the study supervisor. Women with PHQ-9 score ≥10 were referred to nearby health facilities.

Assessment of salivary cortisol was conducted in the first 550 mothers enrolled in Study III. Salivary specimens were collected on day-28 of birth. To account for the diurnal fluctuations and variability due to breastfeeding, we collected the salivary specimens in the morning, before and after breastfeeding, and not later than 1200 hours in the day. The saliva specimens were transported to the laboratory in cold boxes within 4-6 hours of collection and stored at -200C until analysis. To ensure proper transport of specimens to the laboratory, a standard checklist of activities was followed, a specimen transport register, and a lab receiving register was maintained. We estimated the cortisol concentration in the automated 'EVOLISTM Twin Plus' ELISA system (BioRad, California, USA) using the 'Salimetrics® Cortisol Enzyme Immunoassay Kit' (Pennsylvania, USA) at CRL SAS, New Delhi. In our laboratory, the kit performed within acceptable limits for accuracy, precision, and linearity for values between 0.038 μg/dL to 1.0 μg/dL. Specimens with values ≤0.037 μg/dL were assigned the lowest threshold value as per kit instructions.

4.7 Assessment of KMC Practice

The duration of SSC and exclusive breastfeeding was captured by the primary KMC trial outcome ascertainment team at the end of neonatal period and was based on the information provided by the mothers. This team was not involved in the assessment of any of the outcomes of the studies included in the PhD thesis. The interview processes to ascertain SSC and breastfeeding practices were similar for all study participants in the intervention and control arms.

4.8 Training and Quality Control

Prior to initiation, the study teams were trained in Good Clinical Practices and National ethical guidelines for biomedical and health research involving human participants (117). All study teams were briefed on the study including brief background, objectives, implementation strategy, job responsibilities and form filling.

The study teams were further trained in their specific roles and responsibilities. Observation of screening and enrolment in the studies were conducted by coordinators through accompanied visits. Each worker was observed at least once every month. For each enrollment worker, one successful enrollment was observed. For outcomes specific to the studies included in the PhD thesis i.e., breastfeeding performance and maternal postpartum depressive symptoms, the study supervisor made random quality checks in 1% of the assessments.

4.9 Data Management

A local data management centre (DMC) was set up in the field office in Palwal, Haryana. All data related to the studies included in the PhD thesis were collected on paper forms. Double data entry was conducted by data entry operators. Range and logical checks were incorporated to ensure correct data entry. Queries generated were communicated to the study coordinator for resolution within 72 hours and corrections were incorporated. Cleaned and final dataset were stored in a server at the local DMC and was backed up in the password protected central cloud-based central server. The central DMC at CHRD, SAS, had full custody

over the data. The study personnel in the central DMC had user access authorization. The investigator did not have access to the data files before the end of the individual studies.

4.10 Statistical Methods

4.10.1 Sample Size Calculation

For all the studies included in the PhD thesis, the sample sizes were calculated prior to initiation.

For Study I, we assumed that 50% of the infants in the control arm would show effective breastfeeding (118). To detect a minimum of 25% relative change in the proportion of infants with effective breastfeeding with the intervention, with 80% power, 95% confidence, and 10% attrition, the total sample size deemed necessary according to a two-sided test was calculated to be 548 participants.

For Study II, to detect at least a 0.5 SD change in the mean concentration of fecal biomarkers between the trial arms with 95% confidence and 90% power, the total sample size deemed necessary was calculated to be 168 participants based on a two-sided test. Considering, a 15% attrition due to loss to follow-up or failed stool specimen collection or processing the total sample size necessary was 198 participants.

For Study III, we assumed a prevalence of moderate-to-severe postpartum depressive symptoms to be 19% among mothers in the control arm (119). To detect a minimum of 30% relative change with the intervention in the proportion of mothers with moderate-to-severe postpartum depressive symptoms, with 90% power, 95% confidence, and 10% attrition, the total sample size deemed required was 1942 participants based on a two-sided test.

4.10.2 Statistical Analysis

For each of the three studies included in the PhD thesis, a statistical analysis plan was first finalized with the supervisors prior to conducting data analysis. Detailed plans of analysis are mentioned in the individual papers (107-109).

The statistical analysis was conducted using version 16 of STATA (Stata Corporation, College Station, TX, USA) software. Analysis was done on an intent-to-treat basis, i.e., all randomized participants in whom the study primary outcome was recorded were included in the analyses. In the three studies included in the PhD thesis, data were available for 93% or more of the enrolled study participants for the primary study outcomes. Imputation of data points for missing data were not necessary to be conducted in any of the three studies as the losses to follow up were few (120).

All registered participants and their follow-up for each of the three studies included in the PhD thesis were described by study arm in trial profiles. Baseline characteristics were tabulated across the intervention and control arms participants in whom primary outcome assessment was completed in the studies to identify key variables if any, that were unequally distributed across the study arms (a priori defined as a relative difference of >10%). In addition, the distribution of baseline characteristics in all randomized (including those who did not complete follow-up) against all analysed participants were tabulated to appraise external validity. The baseline characteristics of the primary KMC trial along with that of the three studies included in the PhD thesis were tabulated for comparison. Gestational age was considered as per the ultrasonography (USG) reports, when available. If USG was not available, the gestational age was estimated based on the last menstrual period as documented in hospital records or as per maternal recall, whichever was available, in the given order of preference.

To provide insights on intervention compliance, we have described information on SSC practice (number of days and duration per day) and proportion who initiated breastfeeding within 1 hour of birth for each study arm. The number of home visits conducted by government ASHA workers as a part of the routine public health HBNC program in the intervention and control arm participants was also reported.

For reporting of findings, we followed the CONSORT 2010 guidelines (121). CONSORT 2010 guidelines mention that "although the need for adjustment is much less in RCTs than in observational epidemiological studies, an adjusted analysis may be sensible, especially if one or more variables is thought to be prognostic." We presented both unadjusted and adjusted results. Our approach was to avoid overadjustment of non-prognostic variables and avoiding loss of statistical precision (121, 122).

For categorical outcomes, i.e., 'effective breastfeeding performance' and 'moderate-to-severe postpartum depressive symptoms' we estimated the unadjusted relative risk (RR) or prevalence ratio (PR) with 95% confidence interval (CI) using generalized linear models (GLM) of the binomial family with a log link. The statistic PR was used for the outcome 'effective breastfeeding performance' and RR for the outcome 'moderate-to-severe postpartum depressive symptoms'. In the PhD studies, as the outcomes were measured over a short period of time i.e. within 1 month of birth, PR can be interpreted as representing an effect similar to that of RR. We estimated the adjusted RR (aRR) or adjusted PR (aPR) with 95% CI using multivariable GLM of the binomial family with a log link. In the multivariable analysis we included a potential confounding factor for adjustment, only if it was unequally distributed between the study arms at baseline (a priori defined as a relative difference of >10%). Potential confounding factors were defined based on prior knowledge and if associated with the primary outcome at $p < 0.1$ in univariable analysis which included data from both trial arms. For analysis of the secondary outcomes, similar principles were followed (107-109).

For concentration of the fecal biomarkers (continuous variables), given the right-skewed distribution of the data, we used log-transformed (natural logarithm) values for the statistical analyses. To estimate any substantial differences in the unadjusted mean of the log-transformed concentrations of the fecal biomarkers at 1 month of age between the intervention and the control arm, we used Student's t-test. We used multivariable generalized linear models (GLM) of the Gaussian family with an identity link to estimate the adjusted difference in means of the log-transformed fecal biomarkers between the trial arms. In the multivariable model, we accounted for the baseline concentration of the respective fecal biomarkers and included the unequally distributed (a priori defined as a relative difference of >10%) potential confounding factors at

baseline for adjustment. Calculation of the enteric enteropathy (EE) score and index is based on principal component analysis using the myeloperoxidase, neopterin, and alpha-1-antitrypsin concentrations (81). Further details are mentioned in publication 2 of the thesis (108).

LBW infants who are born preterm might respond differently to interventions than those born at term . In the studies included in the PhD thesis, pre-specified sub-group analysis was conducted to estimate whether the effect of the intervention on the respective primary outcomes was different in preterm infants (<37 weeks gestation) compared to term infants (≥37 weeks gestation). To estimate any biological interaction between the intervention and infants being born preterm on categorical outcomes ('effective breastfeeding performance' and 'moderate-to-severe postpartum depressive symptoms'), the absolute excess risk due to interaction (123) using an interaction term in the GLM analysis was reported. A positive value of the absolute excess risk due to interaction with the 95% CI not including zero would indicate that the combined effect is greater than the individual effects signifying presence of biological interaction (123).

4.11 Ethical Approval

Ethics approval was obtained from the Institutional Ethics Review Committee (Ethics Review Committee, Centre for Health Research and Development Society for Applied Studies) and the Regional Committee for Medical and Health Research Ethics (REK) in Western Norway. Trial registration was done in Clinical trials registry-India (CTRI/2017/04/008430).

5 RESULTS

5.1 Approach

The results of the studies contributing to this PhD thesis are presented in the sequence of the objectives. The baseline characteristics of the participants in the three studies are presented first. This is followed by the findings of the effect of promotion and support of KMC on infant breastfeeding performance (Study I), infant gut function (Study II) and maternal postpartum depressive symptoms (Study III). While describing each of the studies, I have presented the results on the primary outcomes, followed by the secondary outcomes.

The loss to follow up was low (less than 7%) in the three studies. For baseline comparisons between the intervention (KMC) and control arms, I have presented the details of participants who completed outcome assessment at the end of follow up and were included in analysis (all analysed participants), for the primary purpose of screening for confounders that are unequally distributed between the intervention and control arm. In addition, for Study I and III, I have presented the distribution of baseline characteristics in all randomized (including those who did not complete outcome assessment at the end of follow-up) against all analysed participants to provide an even fuller picture of the participants and thereby enable readers to better assess external validity. Further, a comparison of the baseline characteristics of the primary KMC trial along with that of the PhD studies are presented (unpublished data).

5.2 Enrolment and Baseline Characteristics

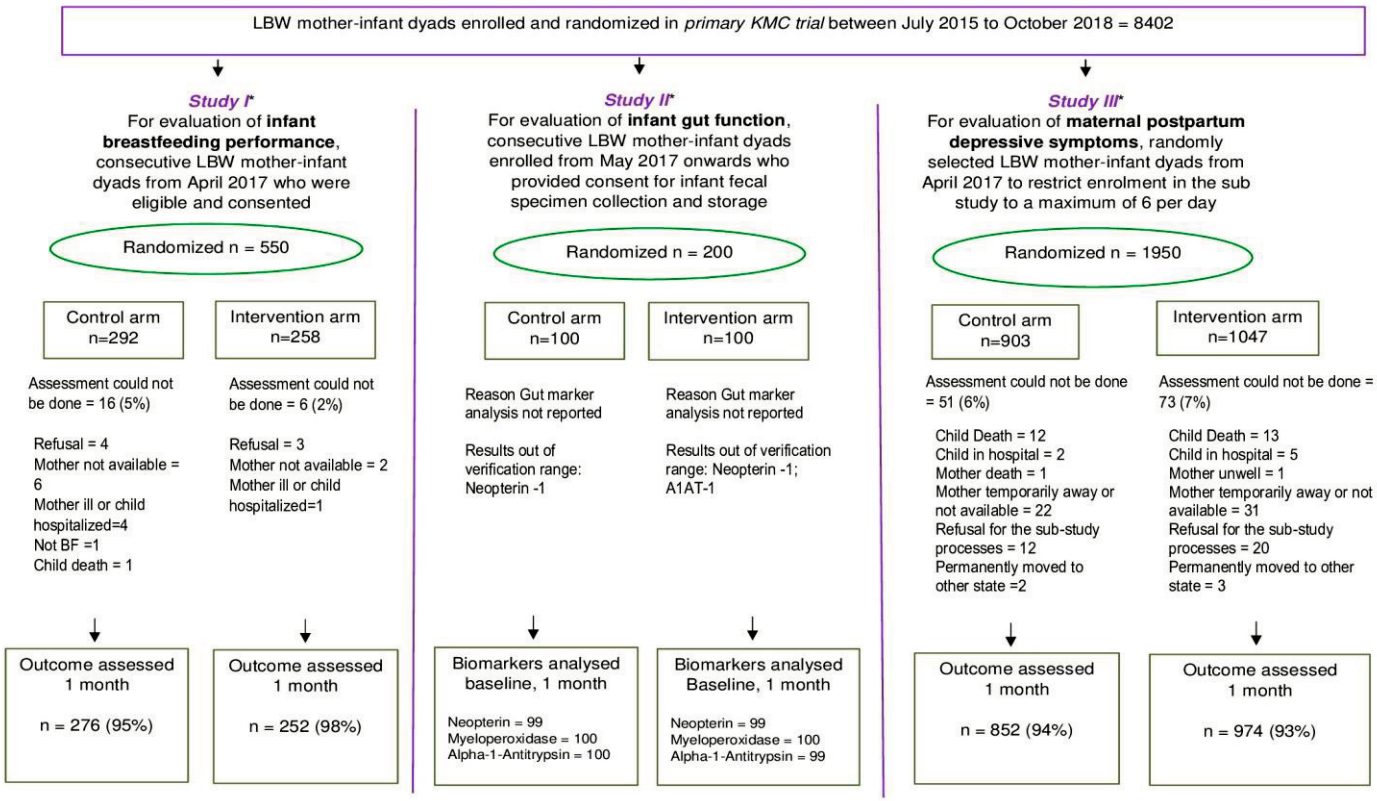
In Study I, for the infant breastfeeding performance assessment, consecutive eligible 550 mother-infant dyads from April 2017 onwards were enrolled. Enrolment was completed in 5 months i.e., August 2017. Among the 550 enrolled participants, outcome assessments were completed in 98% (252/258)

of the infants in the intervention arm and 95% (276/292) of the infants in the control arm (Figure 9).

In Study II, for infant gut function assessment, consecutive 200 eligible infants were enrolled. Enrolment started from May 2017 onwards and was completed in 4 months. Stool specimens were collected from all 200 infants enrolled in the trial at baseline and at the end of the neonatal period. Assessments were completed in 99% to 100% of the infants in both intervention and control arms for all fecal biomarkers of gut function (Figure 9).

In Study III, for assessment of postpartum depressive symptoms, 1950 eligible participants between April 2017 and March 2018 were enrolled. Among them, PHQ-9 assessments were completed in 93% (974/1047) of the mothers in the intervention arm, and 94% (852/903) of the mothers in the control arm (Figure 9).

Figure 9. Trial Profile of the Studies Included in the PhD Thesis



In all the three studies, most of the baseline characteristics in the intervention arm and control arm participants included in analyses were similar. The proportion of female infants in the studies were around 55% and similar across the intervention and control arms. There were some differences in a few variables. In Study I, the relative differences between the study arms exceeded 10% for the variables of home delivery and high birth order (≥5). In Study II, the relative differences between study arms for the variables viz. wealth quintiles (lower 3 quintiles), availability of toilet facility in the household, source of drinking water (public tap), high birth order, and sex of the baby, exceeded 10%. In Study III, the relative differences between study arms for the variable high birth order, exceeded 10% (Table 2). These above-mentioned variables were included in the multivariable analysis of the respective studies for adjustment as they were associated with the outcome.

We did not observe any major differences in the distribution of baseline characteristics between all randomized participants compared to all analysed participants in Study I and III (Supplementary Table 1). There were no major differences in the baseline characteristics of the participants enrolled in the three studies included in the PhD thesis (Study I, II and III) compared to that of the participants in the primary KMC trial (Supplementary Table 2).

Table 2. Baseline Characteristics of the Participants in the PhD Studies

Variables[1]	Study I		Study II		Study III	
	Control arm (N=276) n (%)	KMC arm (N=252) n (%)	Control arm (N=100) n (%)	KMC arm (N=100) n (%)	Control arm (N=852) n (%)	KMC arm (N=974) n (%)
Household characteristics						
Wealth Quintiles						
Least poor	53 (19.2)	60 (23.8)	19 (19.0)	16 (16.0)	146 (17.1)	167 (17.2)
Less poor	61 (22.1)	50 (19.8)	25 (25.0)	27 (27.0)	157 (18.4)	181 (18.6)
Poor	53 (19.2)	49 (19.4)	17 (17.0)	26 (26.0)	178 (20.9)	204 (20.9)
Very poor	58 (21.0)	52 (20.6)	25 (25.0)	13 (13.0)	204 (23.9)	229 (23.5)
Most poor	51 (18.5)	41 (16.3)	14 (14.0)	18 (18.0)	167 (19.6)	193 (19.8)
Median number of family members (IQR)	7 (5 to 9)	7 (5 to 10)	7 (5 to 9)	7 (5 to 10)	7 (5 to 9)	7 (5 to 10)
Maternal characteristics						
Mean (SD) maternal age in years	23.4 (3.7)	23.2 (3.3)	24.1 (3.7)	23.1 (3.2)	23.1 (3.5)	23.4 (3.6)
Maternal education: Median (IQR) years of schooling	7 (0 to 10)	7 (0 to 10)	8 (0 to 12)	6 (0 to 10)	7 (0 to 10)	7 (0 to 10)
Birth related characteristics						
Home delivery	47 (17.0)	36 (14.3)	14 (14.0)	16 (16.0)	129 (15.1)	150 (15.4)
Birth order						
1	96 (34.9)	96 (38.1)	35 (35.0)	37 (37.0)	343 (40.3)	367 (37.7)
2 to 4	151 (54.9)	136 (54.0)	56 (56.0)	56 (56.0)	450 (52.8)	515 (52.9)
≥5	28 (10.2)	20 (7.9)	9 (9.0)	7 (7.0)	59 (6.9)	91 (9.4)
Infant characteristics						
Sex of the baby: Female	147 (53.3)	141 (55.9)	52 (52.0)	59 (59.0)	470 (55.2)	557 (57.2)
Mean (SD) weight at enrolment in gm	2076 (166)	2095 (154)	2095 (162)	2086 (139)	2085 (159)	2089 (160)
Mean (SD) gestational age in weeks[2]	35.6 (2.2)	35.7 (2.0)	35.9 (1.9)	35.9 (1.6)	35.8 (2.0)	35.8 (2.0)
Proportion born preterm (<37 weeks)	178 (64.9)	164 (65.1)	62 (62.0)	63 (63.0)	535 (62.8)	612 (62.8)

[1]In participants who completed outcome assessment. Figures indicate number (%) unless otherwise indicated e.g., mean (SD), median (IQR)

[2]In Study I 65% (360/550), Study II 67.5% (135/200), and in Study III 68.6% (1253/1826), had an ultrasound for gestational age assessment

5.3 Practice of Skin-to-Skin Contact, Breastfeeding, and ASHA Home Visits

Across all the three studies included in the PhD thesis, among the intervention arm participants, the mean time to initiation of skin-to-skin contact (SSC) was 48 hours of birth, and the duration of SSC practice was 28 days with 12 hours per day. There was no substantial difference in the duration of practice of SSC between female or male child. In the control arm, 4-8% of the participants reported practice of SSC. The mean duration of SSC practice in the control arm participants was 1-2 days with half an hour or less per day (Table 3). In the intervention arm, practice of exclusive breastfeeding at 1 month of child age exceeded 80% while it was between 44-60% in the control arm (Table 3).

During the neonatal period, the reported median number of home visits conducted by government ASHA workers was 2 per child in both intervention and control arm infants.

Table 3. Practice of Skin-to-Skin Contact, Breastfeeding, and ASHA Home Visits in KMC and Control Arm Infants

Variables[1]	Study I		Study II		Study III	
	Control arm (N=276) n (%)	KMC arm (N=252) n (%)	Control arm (N=100) n (%)	KMC arm (N=100) n (%)	Control arm (N=852) n (%)	KMC arm (N=974) n (%)
Skin to Skin contact (SSC)						
Any SSC received from enrolment to day 28 of birth	24 (8.7)	252 (100.0)	4 (4.0)	100 (100.0)	33 (3.8)	971 (99.7)
Days with SSC						
Mean (SD)	2.1 (7.1)	27.5 (2.4)	0.7 (4.0)	28.0 (1.0)	(5.2)	27.5 (2.8)
Median (IQR)	0 (0-0)	28 (28-28)	0 (0-0)	28 (28-28)	0 (0-0)	28 (28-28)
Duration of SSC per day in hour						
Mean (SD)	0.5 (2.1)	12.0 (3.8)	0.3 (1.4)	12.2 (3.1)	0.2 (1.4)	12.0 (3.7)
Median (IQR)	0 (0-0)	12 (9-15)	0 (0-0)	13 (10-15)	0 (0-0)	13 (10-15)
Breastfeeding						
Time breastfeeding initiated: hour after birth						
Mean (SD)	4.7 (10.5)	4.4 (9.7)	2.5 (5.2)	2.2 (5.5)	4.4 (10.0)	4.0 (8.6)
Median (IQR)	1 (0-3)	1 (0-3)	1 (0-2)	1 (0-2)	1 (0-3)	1 (0-3)
Exclusive Breastfeeding at 28 days of birth	123 (44.6)	225 (89.3)	60 (60.0)	84 (84.0)	486 (57.0)	859 (88.2)
ASHA home visits per child during the neonatal period as reported by mother: Median (IQR)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)

[1]Figures indicate number (%) unless otherwise indicated e.g., mean (SD), median (IQR)

5.4 Findings: Study I

In Study I, our objective was to evaluate the effect of promotion and support of KMC on effective breastfeeding performance (110) in LBW infants at the end of the neonatal period.

The median (IQR) IBFAT score at enrolment was 11 (9 to 12) both in the intervention arm and in the control arm infants. At the mean age of 28 days, 92% (232/252) of the infants in the intervention and 81% (223/276) in the control arm showed effective breastfeeding performance. The aPR (95% CI) for effective breastfeeding performance adjusted for potential confounding baseline factors and baseline IBFAT score was 1.24 (1.16 to 1.32, Table 4), corresponding to an effect of 24% (16 to 32%); the unadjusted PR was 1.14 (1.06 to 1.22). The absolute unadjusted risk difference (95% CI) was 11.2% (5.5% to 16.9%) and adjusted risk difference (95% CI) was 15.8% (8.6% to 22.9%) higher in the intervention arm. The corresponding number needed to treat for unadjusted and adjusted analysis was 9 infants (6 to 18) and 6 infants (4 to 12), respectively.

In the subgroup of babies born preterm, the adjusted PR (95% CI) of effective breastfeeding performance in the intervention arm was 1.30 (1.18 to 1.42), and that among the subgroup of full-term infants was 1.13 (1.07 to 1.19) (Table 4). Absolute excess risk due to interaction between preterm birth and KMC for the primary outcome was estimated to be 0.05 (95%CI, -0.05 to 0.16), suggesting that the joint effect was marginally higher than the sum of individual effects.

Table 4. Effective Infant Breastfeeding at the End of the Neonatal Period Among KMC and Control Arm Infants

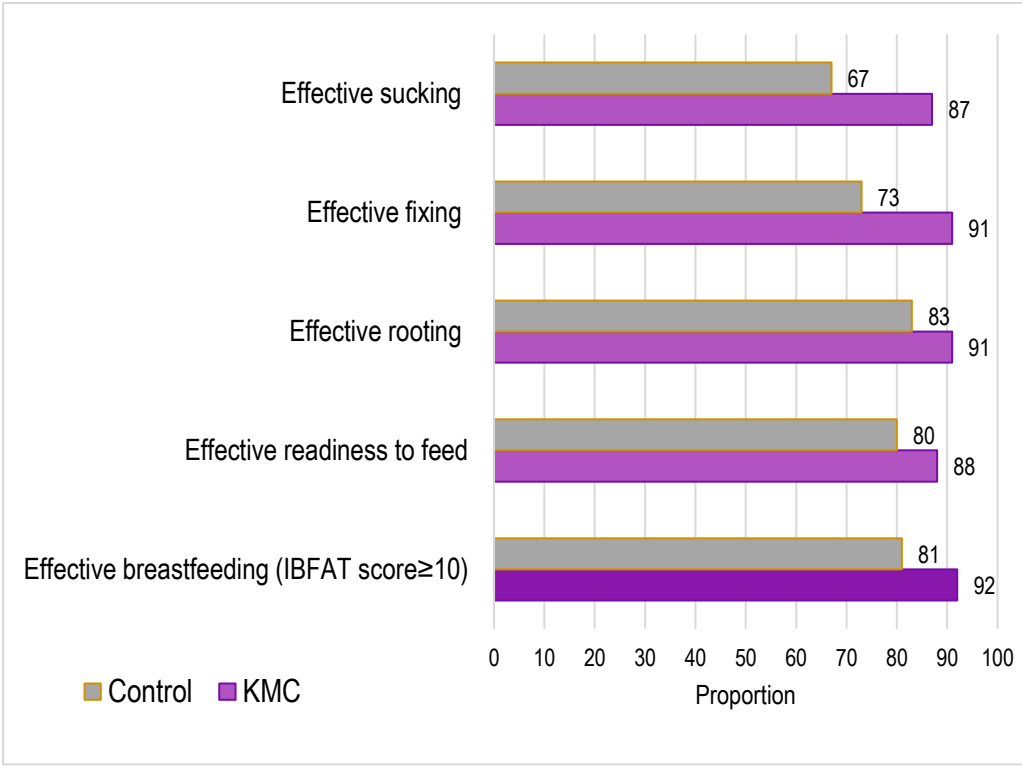
Outcome Variable	Population	Control arm n/N (%)	KMC arm n/N (%)	Unadjusted PR (95% CI)	Adjusted[2] PR (95% CI)
Effective infant breastfeeding (IBFAT[1] score ≥10)	All infants	223/276 (80.8)	232/252 (92.1)	1.14 (1.06 to 1.22)	1.24 (1.16 to 1.32)
	Preterm infants	136/178 (76.4)	147/164 (89.6)	1.17 (1.06 to 1.29)	1.30 (1.18 to 1.42)
	Term infants	87/98 (88.8)	85/88 (96.6)	1.08 (1.00 to 1.18)	1.13 (1.07 to 1.19)

[1]IBFAT=Infant breastfeeding assessment tool

[2]Adjusted for place of delivery, birth order and baseline IBFAT score. The design effect of more than one infant being included from a single household was accounted for by using Stata's cluster option to obtain a robust variance estimator.

A higher proportion of infants in the intervention arm against those in the control arm showed effective readiness to feed (88% vs 80%; aPR 1.13, 95% CI 1.06 to 1.21), effective rooting (91% vs 83%; aPR 1.18, 95% CI 1.12 to 1.25), effective fixing (91% vs. 73%; aPR 1.30, 95% CI 1.21 to 1.40) and effective sucking pattern (87% vs. 67%; aPR 1.32, 95% CI 1.21 to 1.45, Figure 10, Table 5).

Figure 10. Breastfeeding Performance Among Intervention and Control Arm Infants



*Breastfeeding performance was measured using the Infant Breastfeeding Assessment Tool (IBFAT) which has four components i.e., sucking, fixing (latch-on), rooting, and readiness to feed. The IBFAT score is the composite measure of the four components and ≥10 is defined as effective breastfeeding.

Table 5. IBFAT Subcomponent Scores in KMC and Control Arm Infants at the End of Neonatal Period

IBFAT[1] subcomponent score	Control arm n=276 n (%)	KMC arm n=252 n (%)	Unadjusted PR (95% CI)	Adjusted[2] PR (95% CI)
Readiness to feed				
No stimulation needed [3]	221 (80.1)	223 (88.5)	1.10 (1.03 to 1.19)	1.13 (1.06 to 1.21)
Mild stimulation needed [2]	49 (17.7)	26 (10.3)		
More stimulation needed [1]	5 (1.8)	3 (1.2)	Reference3	Reference3
Sleepy, not aroused [0]	1 (0.4)	0 (0.0)		
Rooting				
Immediately [3]	228 (82.6)	229 (90.9)	1.11 (1.03 to 1.19)	1.18 (1.12 to 1.25)
Rooting with coaxing [2]	44 (15.9)	22 (8.7)		
Poor rooting with coaxing [1]	4 (1.5)	1 (0.4)	Reference[3]	Reference[3]
No rooting [0]	0 (0.0)	0 (0.0)		
Fixing (Latch on)				
Immediately [3]	201 (72.8)	229 (90.9)	1.24 (1.15 to 1.35)	1.30 (1.21 to 1.40)
After 3-10 minutes [2]	61 (22.1)	20 (7.9)		
After >10 minutes [1]	13 (4.7)	3 (1.2)	Reference[3]	Reference[3]
Did not start feeding [0]	1 (0.4)	0 (0.0)		
Sucking pattern				
Sucked well [3]	186 (67.4)	219 (86.9)	1.29 (1.17 to 1.42)	1.32 (1.21 to 1.45)
On and off with encouragement [2]	78 (28.3)	30 (11.9)		
Poor or weak sucking [1]	11 (4.0)	3 (1.2)	Reference[3]	Reference[3]
No sucking [0]	1 (0.4)	0 (0.0)		

[1]IBFAT=Infant breastfeeding assessment tool

[2]Adjusted for place of delivery and birth order and the baseline IBFAT subcomponent score. The design effect of more than one infant being included from a single household was accounted for by using Stata's cluster option to obtain a robust variance estimator.

[3]For calculation of the prevalence ratio for the highest score in each subcomponent, scores ≤2 are considered as the reference category

At the end of the neonatal period, the median (IQR) of the IBFAT score was 12 (12 to 12) in the KMC arm and 12 (10 to 12) in the control arm infants. The cumulative frequency plot showed a right shift of the IBFAT score in the intervention arm compared with the control arm (Figure 11), showing that at any given cut-off of the IBFAT score, a higher proportion of infants in the intervention arm was above the cut-off (Table 6).

Figure 11. Cumulative Frequency Plot Showing IBFAT Scores at the End of Neonatal Period

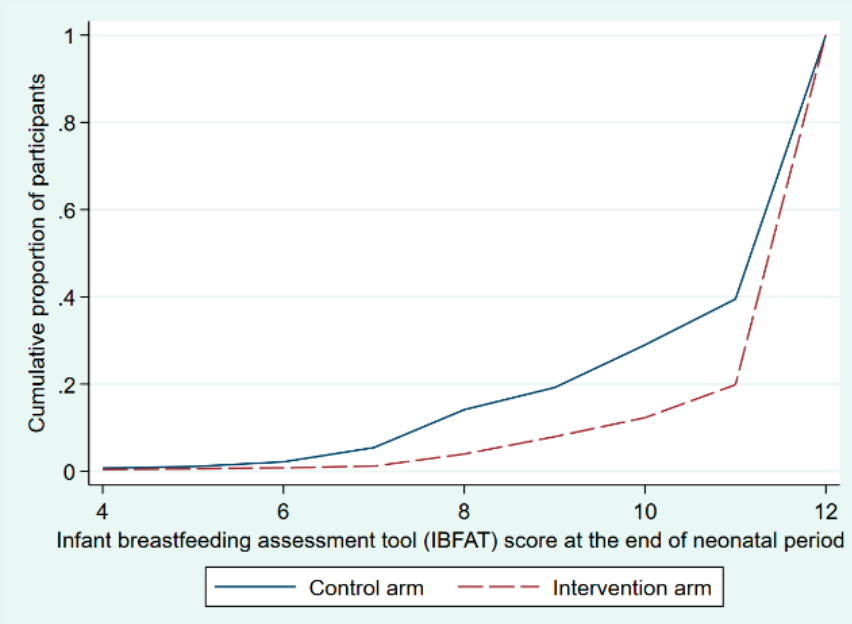


Table 6. Infant Breastfeeding Performance at the End of Neonatal Period by Different Cut-offs of the IBFAT Score

IBFAT[1] cut off score	Control arm N= 276 n (%)	KMC arm N= 252 n (%)	Unadjusted Prevalence Ratio (95%CI)	Adjusted Prevalence Ratio[2] (95% CI)
12	167 (60.5)	202 (80.2)	1.32 (1.18 to 1.49)	1.37 (1.23 to 1.53)
≥11	196 (71.0)	221 (87.7)	1.23 (1.13 to 1.35)	1.32 (1.22 to 1.43)
≥10	223 (80.8)	232 (92.1)	1.14 (1.06 to 1.22)	1.24 (1.16 to 1.32)
≥9	237 (85.8)	242 (96.0)	1.12 (1.06 to 1.18)	1.19 (1.13 to 1.25)
≥8	261 (94.6)	249 (98.8)	1.05 (1.01 to 1.08)	1.10 (1.07 to 1.13)
≥7	270 (97.8)	250 (99.2)	1.01 (1.00 to 1.04)	1.07 (1.05 to 1.09)
≥6	273 (98.9)	251 (99.6)	1.00 (0.99 to 1.02)	1.00 (not estimable)
≥5	274 (99.3)	251 (99.6)	1.00 (0.99 to 1.02)	1.00
≥4	276 (100)	252 (100)	-	-

[1]IBFAT=Infant breastfeeding assessment tool

[2]Adjusted for place of delivery and birth order and baseline IBFAT score. The design effect of more than one infant being included from a single household was accounted for by using Stata's cluster option to obtain a robust variance estimator.

Among the mothers in the KMC arm, 65% (164/252) were very satisfied with their infant's breastfeeding at the end of the neonatal period against 51% (141/276) of the mothers in the control arm. The aPR was estimated to be 1.22 (95% CI 1.05 to 1.41, Table 7).

In the infants randomized to the KMC arm, the proportion of reported EBF at the end of the neonatal period was 89% (225/252) against 45% (123/276) in the infants in the control arm (aPR 1.62, 95% CI 1.45 to 1.81, Table 7). The median (IQR) number of breastfeeds reported per day (24-hour recall) was 12 (12 to 14) in the infants in the KMC arm, and 11 (9 to 14) in the infants in the control arm. The mean (SD) duration of each breastfeeding session was 15.5 (5.1) minutes among the infants in the KMC arm against 10.1 (5.1) minutes among those in the control arm, as per maternal report (mean difference 5.4, 95% CI 4.5 to 6.3).

Table 7. Effect of KMC on Maternal Perception on Infant Breastfeeding and breastfeeding prevalence (24-Hour Recall) at the End of Neonatal Period

Outcomes	Control N=276 n (%)	KMC N=252 n (%)	Unadjusted PR (95% CI)	Adjusted PR (95% CI)
Maternal perception of infant breastfeeding (7-day recall)				
Very satisfied	141 (51.1)	164 (65.1)	1.28 (1.10 to 1.48)	1.22 (1.05 to 1.41)[1]
Satisfied	110 (39.8)	83 (32.9)		
Somewhat satisfied	16 (5.8)	4 (1.6)	Reference[2]	Reference[2]
Not satisfied	9 (3.3)	1 (0.4)		
Breastfeeding (24-hour recall)				
Exclusive	123 (44.6)	225 (89.3)	1.61 (1.43 to 1.80)	1.62 (1.45 to 1.81)[3]
Predominant	81 (29.4)	22 (8.7)	Reference[4]	Reference[4]
Partial	42 (15.2)	5 (1.9)		
No	0 (0.0)	0 (0.0)		

[1]Adjusted for place of delivery, birth order and mother's reported perception on breastfeeding at baseline.

[2]For calculation of the prevalence ratio for 'very satisfied', all other categories were considered as a one group for comparison

[3]Adjusted for place of delivery and birth order

[4]For calculation of the prevalence ratio for exclusive breastfeeding, all other categories are considered as non-exclusive breastfeeding

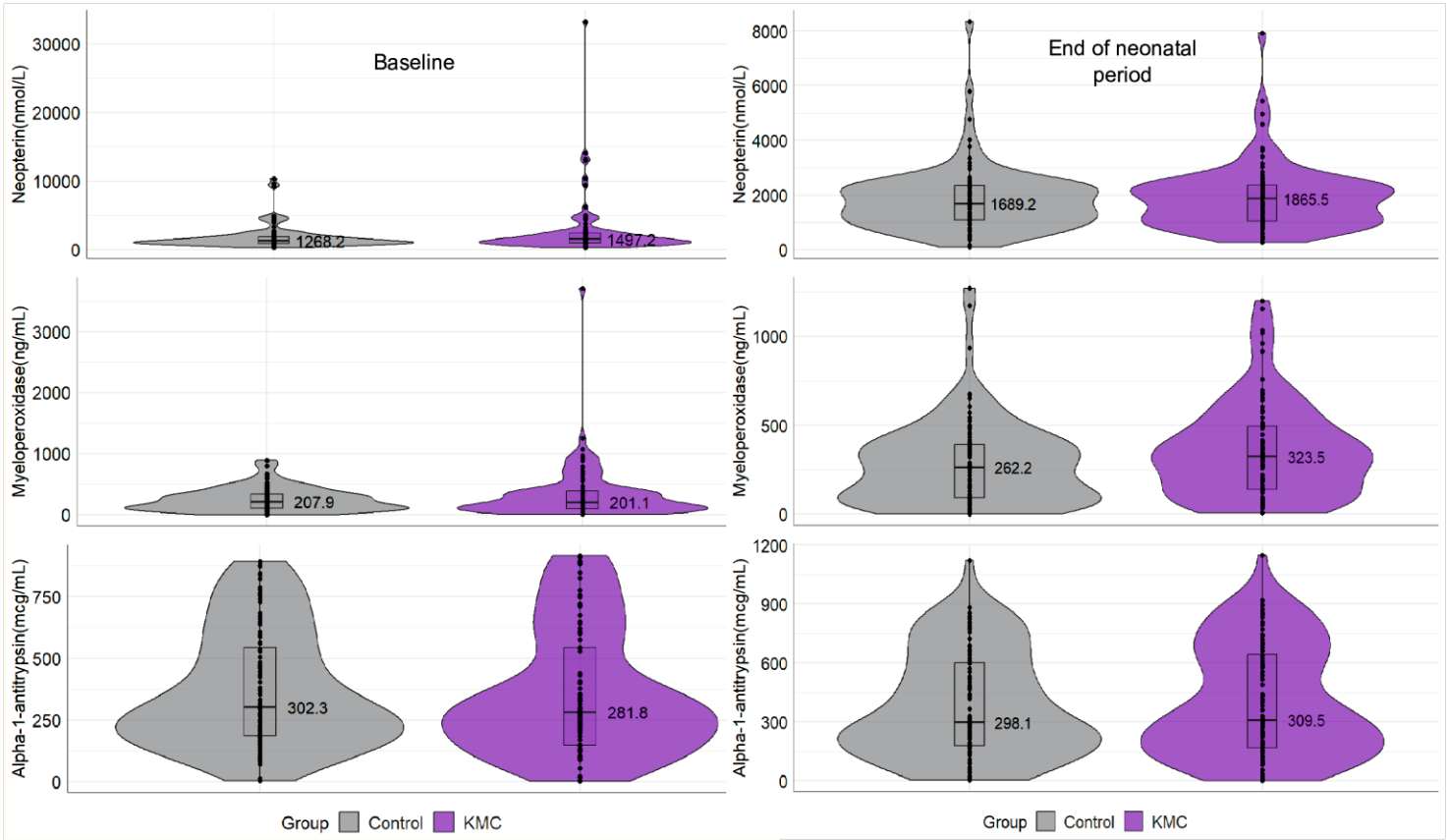
5.5 Findings: Study II

In Study II, our objective was to estimate the effect of promotion and support of KMC in LBW infants on gut function measured by fecal markers of gut inflammation and permeability at the end of the neonatal period.

The mean (SD) age when the baseline stool specimens were collected was 4.2 (1.6) days in the intervention arm and 5.1 (1.2) days in the control arm. The mean (SD) age of stool specimen collection at the end of the neonatal period was 31.6 (3.4) days in the intervention arm and 32.0 (5.5) days in the control arm.

The median (IQR) concentrations of fecal neopterin, myeloperoxidase and alpha-1-antitrypsin at baseline and at the end of the neonatal period in the infants in the KMC arm and those in the control arm were similar (Figure 12). The mean (SD) composite EE score at the end of the neonatal period was 13.6 (7.5) in the intervention arm and 12.4 (8.3) in the control arm infants.

Figure 12. Fecal Biomarkers of Inflammation and Permeability Across Study Arms at Baseline and at the End of the Neonatal Period



The log-transformed mean concentrations of neopterin, myeloperoxidase and alpha-1-antitrypsin were not substantially different among the infants in the intervention arm as compared to those in the control arm. The adjusted difference in means between the intervention and control arm in the log-transformed concentration of neopterin was 0.03 (95% CI -0.15 to 0.21), myeloperoxidase was 0.28 (95% CI -0.05 to 0.61), and alpha-1-antitrypsin was 0.02 (95% CI -0.30 to 0.34). There was no substantial difference in means of the EE score and the EE index either in adjusted or unadjusted analysis. (Table 8).

Table 8. Fecal Biomarker Concentration, Enteric Enteropathy (EE) Score and EE Index Among Study Participants at the End of the Neonatal Period

Fecal biomarkers	Control arm	KMC arm	Unadjusted Difference in means (95% CI)	Adjusted1 Difference in means (95% CI)
Log Neopterin nmol/L				
Mean (SD)	7.30 (0.68)	7.37 (0.61)	0.07 (-0.11 to 0.25)	0.03 (-0.15 to 0.21)
Log Myeloperoxidase ng/mL				
Mean (SD)	5.12 (1.29)	5.49 (1.01)	0.38 (0.05 to 0.70)	0.28 (-0.05 to 0.61)
Log Alpha1antitrypsin µg/mL				
Mean (SD)	5.57 (1.09)	5.61 (1.16)	0.04 (-0.27 to 0.35)	0.02 (-0.30 to 0.34)
EE score				
Mean (SD)	12.36 (8.32)	13.57 (7.51)	1.21 (-1.01 to 3.43)	0.44 (-1.81 to 2.69)
EE index				
Mean (SD)	-0.12 (1.12)	0.12 (0.86)	0.25 (-0.03 to 0.53)	0.17 (-0.11 to 0.45)

1Adjusted for wealth quintiles, toilet facility, source of drinking water, birth order, sex of the baby, and baseline concentration of the respective gut inflammatory markers.

Among the subgroup of term infants, there was no substantial difference between the study arms in the mean log-transformed concentrations of fecal biomarkers, nor in the EE score or the EE index (Table 8). In the subgroup of preterm infants, the log-transformed mean concentration of myeloperoxidase and the EE index was somewhat higher in the infants in the intervention arm against control arm. In preterm infants, the adjusted difference in means between study arms in the log-transformed concentration of myeloperoxidase was estimated to be 0.41 (95% CI 0.02 to 0.82), and that for the EE index was 0.38 (95% CI 0.01 to 0.75, Table 9).

Table 9. Effect of KMC on Infant Fecal Biomarkers at the End of the Neonatal Period in Subgroups of Preterm and Term Infants

Gut Biomarkers at 1 month	Infant subgroup	Control arm Mean (SD)	KMC arm Mean (SD)	Unadjusted Difference in means (95% CI)	Adjusted[1] Difference in means (95% CI)
Log Neopterin nmol/L	Preterm (n=125)	7.27 (0.75)	7.43 (0.61)	0.17 (-0.07 to 0.41)	0.16 (-0.09 to 0.40)
	Term (n=75)	7.37 (0.57)	7.27 (0.60)	-0.10 (-0.37 to 0.17)	-0.26 (-0.55 to 0.04)
Log Myeloperoxidase ng/mL	Preterm (n=125)	5.13 (1.13)	5.51 (0.98)	0.39 (0.01 to 0.76)	0.41 (0.02 to 0.82)
	Term (n=75)	5.11 (1.54)	5.47 (1.09)	0.36 (-0.25 to 0.98)	0.01 (-0.58 to 0.60)
Log Alpha1antitrypsin µg/mL	Preterm (n=125)	5.39 (1.24)	5.55 (1.23)	0.16 (-0.28 to 0.60)	0.24 (-0.21 to 0.69)
	Term (n=75)	5.86 (0.74)	5.70 (1.02)	-0.16 (-0.57 to 0.25)	-0.21 (-0.66 to 0.24)
EE Score at 1 month	Preterm (n=125)	11.7 (7.9)	14.2 (7.6)	2.5 (-0.27 to 5.26)	2.5 (-0.40 to 5.36)
	Term (n=75)	13.5 (8.9)	12.5 (7.2)	-0.99 (-4.77 to 2.80)	-3.84 (-7.76 to 0.08)
EE index at 1 month	Preterm (n=125)	-0.18 (1.1)	0.19 (0.8)	0.37 (0.02 to 0.72)	0.38 (0.01 to 0.75)
	Term (n=75)	-0.04 (1.1)	0.01 (0.8)	0.04 (-0.43 to 0.51)	-0.29 (-0.76 to 0.18)

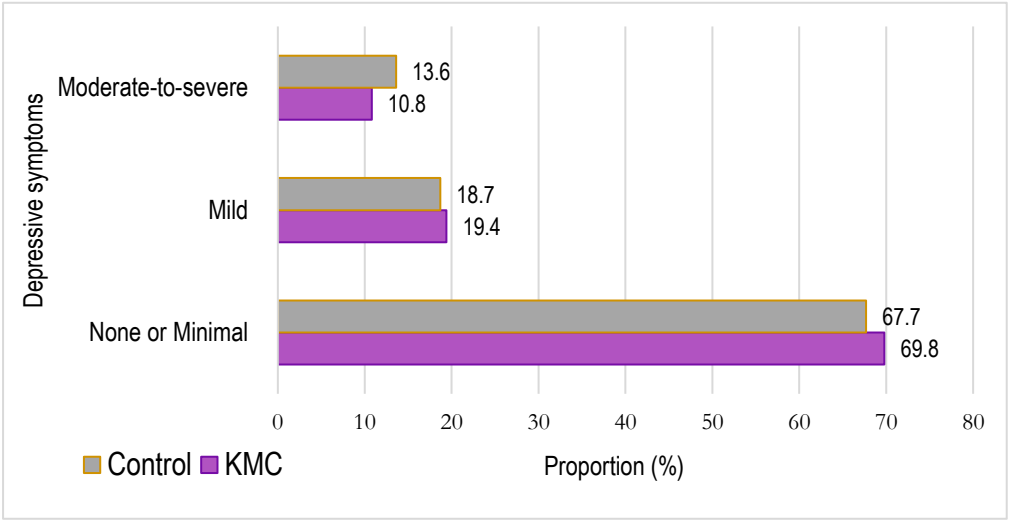
[1]Adjusted for wealth quintiles, toilet facility, source of drinking water, birth order, sex of the baby, and baseline concentration of the respective gut inflammatory markers.

5.6 Findings: Study III

In Study III, our objective was to estimate the effect of promotion and support of KMC in LBW infants on the risk of maternal postpartum depressive symptoms at 28 days after birth.

The proportion (95% CI) of mothers with moderate-to-severe postpartum depressive symptoms (PHQ-9 score ≥10) was 10.8% in the intervention arm against 13.6% in the control arm (Figure 13).

Figure 13. Proportion (%) of Mothers with Depressive Symptoms in the KMC and Control Arms of the Trial



The RR (95% CI) for moderate-to-severe postpartum depressive symptoms in the intervention arm against control arm, adjusted for birth order categories and taking household clustering into account, was 0.75 (0.59 to 0.96, Table 10). The corresponding efficacy of the intervention was 25% (4% to 41%). The unadjusted RR for moderate-to-severe postpartum depressive symptoms in the intervention arm was 0.79 (0.62 to 1.01). The unadjusted absolute risk difference (95% CI) for moderate-to-severe postpartum depressive symptoms was 2.8% (0.1% to 5.8%) and the adjusted absolute risk difference (95% CI) was 3.1% (0.3% to 6.3%) lower in the intervention arm. The corresponding number needed to treat (95% CI) for unadjusted and adjusted analysis was estimated to be 36 (17 to 1000) and 32 (16 to 333), respectively. This suggests that supporting 32 mothers to provide KMC can avoid one mother getting postpartum depressive symptoms.

In the sub-group of mothers with preterm births, the RR (95% CI) of moderate-to-severe postpartum depressive symptoms in the intervention arm was 0.71 (0.52 to 0.96) as against 0.86 (0.56 to 1.34) in the subgroup of mothers with term babies. The absolute excess risk due to interaction (AERI) for preterm birth and KMC on maternal postpartum depression was -0.03; 95% CI, -0.09 to 0.03, suggesting that the joint effect of KMC promotion and preterm birth is not substantially higher than the sum of the individual effects.

The RR (95% CI) for any depressive symptoms (PHQ-9 score ≥5) in the intervention arm adjusted for birth order categories and taking household clustering into account was 0.92 (0.81 to 1.05); the unadjusted RR was 0.94 (0.82 to 1.07, Table 10). Analysed another way, multinomial regression showed a similar adjusted effect size of the intervention on the risk of moderate-to-severe depressive symptoms (RR, 0.73; 95% CI, 0.54 to 0.98), but no effect on mild depressive symptoms (RR, 1.00; 95% CI, 0.79 to 1.26).

Table 10. Effect of KMC on Maternal Postpartum Depressive Symptoms Among Study Participants

Outcome Variable	Population	Control arm n/N (%)	KMC arm n/N (%)	Unadjusted RR (95% CI)	Adjusted[1] RR (95% CI)
Moderate-to-severe depressive symptoms (PHQ9≥10)	All mothers	116/852 (13.6)	105/974 (10.8)	0.79 (0.62 to 1.01	0.75 (0.59 to 0.96)
	Subgroup – Mothers of Preterm infants	80/535 (15.0)	68/612 (11.1)	0.73 (0.55 to 1.00	0.71 (0.52 to 0.96)
	Subgroup – Mothers of Term infants	36/317 (11.4)	37/362 (10.2)	0.90 (0.58 to 1.39	0.86 (0.56 to 1.34)
Any depressive symptoms (PHQ9≥5)2	All mothers	275/852 (32.3)	294/974 (30.2)	0.94 (0.82 to 1.07	0.92 (0.81 to 1.05)
	Subgroup – Mothers of Preterm infants	183/535 (34.2)	195/612 (31.2)	0.74 (0.55 to 1.00	0.92 (0.78 to 1.09)
	Subgroup – Mothers of Term infants	92/317 (29.0)	99/362 (27.3)	0.90 (0.58 to 1.38	0.91 (0.72 to 1.15)

1Adjusted for birth order categories and accounting for household clustering.

2159/852 (18.7%) and 189/974 (19.4%) had mild depressive symptoms in the control and ciKMC arm, respectively

The median (IQR) PHQ-9 scores were 2 (0 to 5) in the intervention arm and 2 (0 to 6) in the control arm mothers. The cumulative frequency plot showed a left-shift of the PHQ-9 scores among intervention arm compared to the control arm mothers (Figure 14), suggesting that at any given PHQ-9 score cut-off, a lower proportion of mothers in the intervention arm were above the cut-offs as compared to the mothers in the control arm (Table 11).

Figure 14. Cumulative Frequency Plot Showing PHQ-9 Scores of the Intervention and Control Arm Mothers

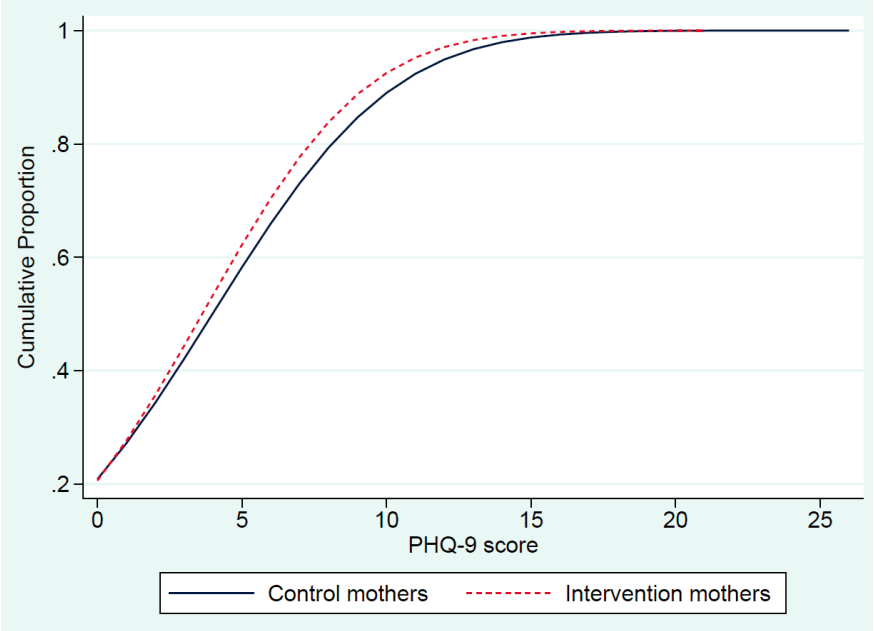


Table 11. Proportion of Mothers with Postpartum Depressive Symptoms in Control and KMC Arm using Different PHQ-9 Cut-off Scores

PHQ-9 cut off score	Control arm N (%)	KMC arm N (%)	Unadjusted Relative Risk[1] (95%CI)	Adjusted Relative Risk[2] (95% CI)
≥2	482 (56.6)	540 (55.4)	0.98 (0.90 to 1.06)	0.97 (0.90 to 1.05)
≥4	340 (39.9)	366 (37.6)	0.94 (0.84 to 1.06)	0.93 (0.83 to 1.04)
≥6	229 (26.9)	242 (24.8)	0.92 (0.80 to 1.08)	0.91 (0.78 to 1.06)
≥8	171 (20.1)	165 (16.9)	0.84 (0.69 to 1.02)	0.82 (0.68 to 0.99)
≥10	116 (13.6)	105 (10.8)	0.79 (0.62 to 1.01)	0.75 (0.59 to 0.96)
≥12	84 (9.9)	76 (7.8)	0.79 (0.59 to 1.06)	0.75 (0.56 to 1.02)
≥14	53 (6.2)	41 (4.2)	0.68 (0.45 to 1.00)	0.63 (0.42 to 0.94)
≥16	33 (3.9)	30 (3.1)	0.80 (0.49 to 1.29)	0.73 (0.45 to 1.20)
≥18	21 (2.5)	15 (1.5)	0.62 (0.32 to 1.20)	0.58 (0.30 to 1.14)
≥20	14 (1.6)	7 (0.7)	0.43 (0.18 to 1.08)	0.40 (0.16 to 1.00)
≥22	3 (0.4)	0 (0.0)	-	-
≥24	3 (0.4)	0 (0.0)	-	-
≥26	1 (0.1)	0 (0.0)	-	-

[1]Unadjusted risk of having higher PHQ-9 scores (based on the given cut-off) in intervention arm mothers compared to control arm mothers.

[2]Adjusted for birth order categories and accounted for household clustering.

6 DISCUSSION

This chapter discusses the study findings in comparison with the available scientific evidence on the topic. The key findings are described first, followed by discussion on strengths and weaknesses. While describing strengths and weakness, I have discussed on the possible biases and the measures taken to minimize them in the studies, wherever possible. At the end of this section, I have interpreted the study findings in context of the target population. Next, the study findings are compared and discussed in background of the existing evidence with a focus on the South Asian settings in the order of the study aims. This section then connects to the scientific conclusions and public health implications in the next chapters.

6.1 Key Findings

The studies included in the PhD thesis conducted in low-middle income neighborhoods in India aimed to study the effect of kangaroo mother care promotion and support in stable LBW infants during the neonatal period on infant breastfeeding performance, infant gut function and maternal postpartum depressive symptoms.

The first hypothesis was that KMC promotion improves effective breastfeeding performance in LBW infants. In the 550 infants included in the study, it was observed that promotion and support of KMC was associated with substantially higher effective breastfeeding performance. The findings suggested that promoting KMC in six LBW mother-infant dyads would result in one more infant breastfeeding effectively. Effective performance in all 4 components of the IBFAT, i.e., readiness to feed, rooting, fixing, and sucking pattern were found to be substantially higher in the infants randomized to the intervention against control arm. In addition, promotion and support of KMC was associated with higher maternal satisfaction with their infant's breastfeeding performance, increased proportion of infants exclusively breastfed at the end of the neonatal

period, with a higher number of breastfeeds per day and longer duration of each breastfeed. Sub-group analysis indicated a possible higher effect of KMC promotion on effective breastfeeding performance in preterm infants than in the term infants.

The second hypothesis was that KMC promotion reduces gut inflammation and permeability as reflected in a lower concentration of fecal neopterin, myeloperoxidase, and alpha-1-antitrypsin and enteric enteropathy as reflected in a lower composite EE score and EE index. In the 200 infants included in the study, there was no association between promotion and support of KMC on the concentrations of any of the fecal biomarkers, overall or among preterm infants.

The third hypothesis was that KMC promotion can reduce the risk of moderate-to-severe maternal postpartum depressive symptoms. In 1950 mothers included in the study, we found that promotion and support of KMC was associated with a substantial lower relative risk of maternal moderate-to-severe postpartum depressive symptoms. The findings showed that promoting KMC in 32 stable LBW mother-infant dyads would prevent one mother from getting moderate-to-severe postpartum depressive symptoms. Sub-group analysis indicated a possible higher effect of KMC promotion on risk reduction of moderate-to-severe maternal postpartum depressive symptoms in mothers of preterm infants than in the term infants.

6.2 Strengths and Weakness of the Studies Included in the PhD Thesis

The key strengths of the studies were the randomized controlled trial design, low and balanced losses to follow-up across study arms, high compliance to intervention promotion, extensively trained and independent outcome assessment teams, rigorous processes for laboratory analysis including use of automated ELISA, pre-determined sample sizes and statistical analysis plans.

In the studies included in the PhD thesis, the likelihood of selection or confounding bias is low due to successful randomization as demonstrated by the similar baseline characteristics in the intervention and control arm. Moreover, the similarity in the baseline characteristics of the studies included in the PhD

thesis with the primary KMC trial reaffirms the representativeness of the same underlying population. The possibility of a biased estimate due to loss to follow-up (124) is very minimal given the low and balanced loss to follow-up across the study arms in the PhD studies, and no substantial differences in the baseline characteristics in all participants analysed against all participants randomized (applicable for Study I and II).

In intervention studies, underestimation of effect could be an issue in case of poor compliance to intervention leading to reduced contrast between study arms (125). In the studies included in the PhD thesis, high compliance to KMC promotion is demonstrated by almost all participants in the intervention arm reporting practice of SSC with a mean duration of 28 days and 12 hours per day, while the participants in the control arm reported no or very minimal SSC practice. Therefore, the issue related to underestimation of the intervention effect is not applicable in the PhD studies.

To minimize measurement errors related to laboratory assessment (salivary cortisol and fecal biomarkers of gut function) we followed standard protocols for specimen collection, transport, and storage. All specimens were transported in cold boxes within 6 to 8 hours of collection and stored in the laboratory at recommended temperature under temperature monitoring systems. To minimize human errors in laboratory testing, we followed standard kit instructions, and used an automated ELISA system for assessment of the biomarkers.

To minimize the possibility of information or misclassification bias in assessment of maternal depressive symptoms and infant breastfeeding performance outcomes, we used questionnaires (PHQ-9 and IBFAT, respectively) that were validated for measurement of the same (115, 126), and previously tested in Indian settings (Hindi language) (111, 127). Nonetheless, some degree of misclassification bias may be possible associated with the use of a single cut-off score, even though the scales are validated. To examine any such possibility, we presented the results using different possible cut-offs for both the questionnaires (in decreasing order for IBFAT and increasing order for PHQ-9). The consistency in the directionality of the effect along with the observed gradient of effect sizes across the different cut-offs, reconfirms validity of the observed results on the primary outcomes. Furthermore, to minimize inter-rater variability among the workers of the outcome assessment team, standardization exercises

were conducted prior to study assessments, and the workers were trained repeatedly.

In well conducted randomized trials, the likelihood of imbalances in the baseline characteristics across trial arms arising out of chance is minimal, but not impossible (128). Such imbalances in the prognostic baseline variables could lead to confounding bias. In the statistical analyses of the studies included in the PhD thesis, a pre-planned adjusted analysis was presented in addition to the unadjusted analysis, as per the CONSORT guidelines (129). This approach accounts for any chance imbalances in the prognostic baseline covariates to get robust estimates of the effect sizes while avoiding loss of power due to overadjustment (122). Moreover, the similarity in the findings of the unadjusted and adjusted estimates indicates no major imbalance in the prognostic baseline covariates. Similar effect size estimates obtained from the different analytical approaches e.g., univariable vs. multivariable regression methods, binomial vs. multinomial regression methods, reaffirms reliability and validity of the findings. While sample sizes were calculated a priori, it is to be noted that our study was not powered to assess less than 0.5 SD change in the mean concentration of fecal biomarkers between the trial arms.

The unmasked nature of the study could have led to observer's bias in measurement. KMC being a behavioural intervention, masking was not possible. In the studies included in the PhD thesis, the outcomes were assessed was by an independent trained team not involved in intervention delivery, after completion of 28 days of life. Only approximately 2% of the mothers continued SSC beyond the 28-day period. Therefore, the possibility of a substantial observer bias to affect the study findings is unlikely.

As determination of the duration of SSC practice and some of the outcomes were based on interview (maternal postpartum depressive symptoms, maternal satisfaction related to infant breastfeeding), the information could be subject to recall bias and possibly affect the validity of the results. Among participants in the intervention arm in the PhD studies, the information on the duration of SSC captured by the outcome assessment team was found to be similar to that obtained by the intervention delivery team, suggesting a low likelihood of recall bias (16). Moreover, given the short recall periods, it is unlikely that the outcomes

of maternal postpartum depressive symptoms (14 days) and maternal satisfaction related to infant breastfeeding (7 days), are subject to substantial recall bias.

Considering the strengths of the study and the measures to address possible weaknesses, the overall risk of bias is low. Therefore, the findings of the PhD studies are likely to be valid, reliable, and representative of the target study population of stable LBW infants. The findings show that in low-middle income populations in Haryana, North India, promotion and support of KMC in LBW infants can substantially improve infant breastfeeding performance, reduce the risk of maternal moderate-to-severe postpartum depressive symptoms, but does not have any substantial effect on the measured fecal biomarkers of infant gut function.

6.3 Comparison with Existing Evidence

Here, I have compared the results of the studies included in the PhD thesis with existing literature (published by 1st April 2023 using the search strategy in Table 1) in order of the objectives.

6.3.1 Kangaroo Mother Care and Infant Breastfeeding

Researchers have evaluated the effect of KMC on exclusive breastfeeding in LBW or preterm infants. But there seem to be lack of randomized trials that had evaluated the effect of KMC on breastfeeding performance or maternal perception of breastfeeding in LBW or preterm infants. However, the effect of SSC practice on breastfeeding performance in healthy term infants in hospital settings had been evaluated earlier.

The findings of the previous hospital-based studies in South Asian countries and other high and low-middle income settings showed that practice of SSC in healthy term infants was associated with improved breastfeeding performance and are similar to the PhD study results. In 2007, researchers from Tennessee, reported a substantially higher IBFAT score in 20 full-term infants randomized to practice SSC compared to the infants in the control arm (20). Findings form a hospital-based randomized trial during 2009-11 in North India that included

298 healthy term infants showed a higher IBFAT score at 6 weeks of age in infants who were supported to practice SSC for at least 2 hours compared to the infants in the control arm (111). In 2014, authors from Iran reported that among 90 term infants, those randomized to practice SSC for 1 hour after birth had higher effective readiness to feed, effective sucking, effective latching and effective rooting (130). In 2019, researchers summarized findings on breastfeeding performance based on IBFAT scores in a meta-analysis that included 3 randomized trials conducted in India, Italy, and the USA. The findings of the meta-analysis suggested that healthy term infants practicing SSC in hospital settings have higher mean IBFAT scores (MD 1.5, 95% CI 1.2 to 1.8) compared to infants with routine care (131).

Evidence from previous studies in South Asian settings on the effect of SSC on maternal perception of breastfeeding in healthy term infants are consistent with the PhD study findings. In 2014, authors from North India, reported that the score of maternal perception about their infant's breastfeeding measured using the 4-point Likert scale, was substantially higher in term infants randomized to SSC arm compared to controls. Among the mothers in the SSC arm 29% were very satisfied with their infants breastfeeding compared to 3% in the control arm (111).

Previous research findings show that KMC can improve exclusive breastfeeding rates in LBW infants similar to our study results. In a meta-analysis in 2016 that included 5 hospital-based randomized trials from low-middle income settings of South Asia (India), Southeast Asia (Indonesia), and other countries (Ecuador, Ethiopia, Mexico), authors reported a 1.2 times higher exclusive breastfeeding rate in LBW infants in the KMC group compared to the control group at 1 to 3 months of age (51).

The above-mentioned previous trials on breastfeeding performance were conducted in a different population of healthy term infants and the duration of SSC practice was lesser. Nonetheless, the findings of the previous studies are in agreement with the results of the PhD study and support that promotion of KMC can substantially improve effective breastfeeding performance, maternal satisfaction related to breastfeeding, and EBF in LBW infants. Biologically, the effect of KMC on effective breastfeeding performance as well as improved maternal satisfaction is plausible. The observed effect may be explained by the

enhanced bi-directional feedback mechanisms between the mother and the child, better bonding, reduced stress, and improved maternal confidence to breastfeed (132).

6.3.2 Kangaroo Mother Care and Infant Gut Function

Researchers have evaluated the effect of different interventions including WASH (water, sanitation, and hygiene) or improved infant feeding practices on infant gut function. However, I did not find any previous studies that specifically evaluated the association of KMC with infant gut function. Our study to my knowledge is the first randomized trial that examined the effect of promotion of KMC on biomarkers of gut inflammation and permeability.

Available evidence from previous randomised trials indicated no clear effect of improved infant feeding practices or WASH interventions on infant gut inflammation or permeability (82, 133). Findings from a trial in Bangladesh, South Asia, did not show any evidence of an effect of age-appropriate nutrition counselling plus a lipid-based nutrient supplement on fecal biomarkers of infant gut inflammation (myeloperoxidase and neopterin) and gut permeability (alpha-1-antitrypsin levels and lactulose-mannitol ratio) in children between 3 to 14 months of age (133). In a cluster-randomized trial in low-middle income settings in Zimbabwe, investigators reported no substantial effect of WASH or infant and young child feeding related interventions on fecal biomarkers of infant gut inflammation (myeloperoxidase and neopterin) and gut permeability (alpha-1-antitrypsin) levels in the first 6 months of life (82).

Although, the interventions evaluated previously are different from that of the PhD study, it is important to note that none of the trials showed evidence of any effect on the identified fecal biomarkers of infant gut inflammation and gut permeability. Some reasons for not observing any differences in the markers of gut inflammation or permeability in our study could be attributed to factors such as a large proportion of control arm participants practicing breastfeeding, and short observation period. While the reported findings may be correct, an alternative explanation might be that the identified fecal biomarkers of infant gut inflammation and gut permeability are not sensitive to assessing response to the intervention on infant gut function. Moreover, it is important to highlight that

EED is a broader syndrome encompassing several aspects of gut function beyond gut inflammation and altered gut permeability, presence of multiple viral and bacterial pathogens, and systemic inflammation (134, 135). Therefore, further research and longer follow-up studies are warranted to clarify the effect of KMC on EED.

6.3.3 Kangaroo Mother Care and Maternal Postpartum Depressive Symptoms

The association between KMC practice in preterm or LBW infants with maternal postpartum depressive symptoms have been evaluated previously by researchers in hospital-based studies. Majority of the previous studies have reported the mean scores on a continuous scale using various assessment tools for depression. In the PhD study, our primary outcome was moderate-to-severe postpartum depressive symptoms. The choice of the primary outcome was driven by its clinical importance. Women with a PHQ-9 score >10 are known to have an increased probability of major clinical depression that needs medical attention, whereas the milder symptom categories are often self-limiting (114).

The findings of the previous hospital-based studies (99-101) conducted in South Asian countries and other high and low-middle income settings suggest a beneficial effect of KMC in mothers of LBW or preterm infants on reduction of maternal postpartum depressive symptoms similar to the PhD study findings. In a recent meta-analysis in 2023, authors reported a 24% relative reduction in the pooled risk of moderate-to-severe postpartum depressive symptoms in mothers of LBW or preterm infants who practiced KMC (3 trials, 4399 participants) (136). The pooled mean score for postpartum maternal depressive symptoms was reported to be lower among mothers in the KMC group versus the control group (SMD: −0.22; 95% CI: −0.47 to 0.02, 11 trials, 3000 participants), but the confidence intervals were imprecise. These findings are in agreement to the results of the Study III. The findings support that while KMC has a substantial beneficial effect on the more severe spectrum of maternal depressive symptoms, it may not have a similar effect on the milder spectrum.

Biologically the effect of KMC in reduction of postpartum depressive symptoms may be explained by improved mother-infant bonding and complex

physiological mechanisms, potentially through enhancement of the oxytocin release (137). Mothers with prolonged separation from their infants due to admission in NICU or other issues have higher likelihood of developing negative emotions like despair, reduced maternal confidence and competence (138, 139). KMC provides an opportunity for mother and infant to be in close contact, which helps the mother to build self-confidence in caring for their premature or LBW infant (140-142), while lowering the risk of maternal depressive symptoms.

Findings from previous studies and meta-analysis conducted in high and low-middle income countries show that the effect of SSC on maternal stress using salivary cortisol as a biomarker can vary (143). In 2005, researchers from Sweden, reported a reduction in the levels of maternal salivary cortisol following SSC sessions in a before-after experimental study design that included 17 preterm infants in neonatal intensive care settings (144). In 2012, researchers from Canada evaluated the effect of SSC on mean salivary cortisol concentrations among mothers of term infants in a quasi-experimental study. The authors reported that the mean salivary cortisol concentrations at the end of neonatal period, among mothers practicing SSC and the control mothers were almost identical. No substantial differences were observed between the groups, which is in agreement with our study findings (98).

Salivary cortisol, although widely used to capture short-term fluctuations in physiological stress, does not seem to be a good indicator for chronic stress (145). In mothers during the postpartum period, because of infant care related stimulations the hypothalamic-pituitary axis may get activated and may lead to acute short-term fluctuations in the salivary cortisol levels. These short-term fluctuations may not necessarily reflect maternal mental stress. A biomarker less amenable to fluctuations like hair cortisol might be a better biomarker for measurement of chronic stress (145, 146).

7 SCIENTIFIC CONCLUSIONS

In low-middle income populations in India or similar settings in South Asia,

1. Promotion and support of KMC practice in stable LBW infants substantially improves effective breastfeeding performance at the end of the neonatal period.

2. Promotion and support of KMC practice in stable LBW infants has no evidence of any substantial effect on fecal biomarkers of infant gut function at the end of the neonatal period.

3. Promotion and support of KMC practice in stable LBW infants substantially reduces the risk of maternal postpartum depressive symptoms at 28 days after birth.

8 PUBLIC HEALTH IMPLICATIONS AND FUTURE RESEARCH NEEDS

This PhD study generated new evidence on effect of KMC on important biological outcomes and adds value to the existing literature (51, 147). The benefits of KMC on improving effective infant breastfeeding performance and reducing maternal depressive symptoms are helpful to explain its clinical benefits on infant survival. The study also adds new knowledge indicating that KMC may not have a measurable effect on the fecal biomarkers of gut inflammation and permeability and underscores the need for further research in this domain.

In 2022, WHO has updated the guidelines for care of LBW or preterm infants and recommends that "KMC can be initiated in the health-care facility or home and should be given for 8-24 months per day (as long as possible)"(14). The findings of the PhD studies are in alignment with the WHO 2022 recommendations and support promotion of KMC among LBW infants in public health programs in low-middle income settings in India and similar South Asian countries. The studies demonstrated that initiation and continuous practice of KMC is possible at home with support from community-level health workers in low-middle income communities. Although KMC is known to improve survival in the preterm and LBW infant (51, 147), its benefit to the mother was less clear and a perceived barrier to KMC promotion (55). The PhD study on postpartum depressive symptoms addresses an important knowledge gap in this aspect. The evidence supports KMC promotion as an intervention to improve maternal mental health in the postnatal period. The study findings on improved effective infant breastfeeding, supports integration of KMC within the essential newborn care programs in low-middle income communities in South Asia. Moreover, previous literature suggests that the KMC intervention does not seem to increase inequity in survival, with a tendency of higher impact among those belonging to lower wealth quintiles or whose mothers were less educated (148). KMC has little demand for household resources beyond mother's or caregivers time, does not require high technical expertise, and is culturally acceptable (56). These characteristics may facilitate adoption of KMC in low-income households.

The findings of the PhD studies might have some clinical implications. In low-middle income settings in India, caregivers of LBW infants often visit the primary health care centres or local physicians to seek medical help for difficulties in latching, sucking, nipple-related problems, and poor breastfeeding (149-151). In addition, in low-middle income countries in the Indian subcontinent (South Asia), around one-fifth of the mothers suffer from postpartum depression and needs clinical attention (96, 119). The evidence from the PhD studies supports that practice of KMC can be offered as a potential solution to ameliorate the difficulties associated with breastfeeding in LBW infants and to prevent maternal depressive symptoms in the postpartum period.

The studies included in the PhD thesis had some limitations. First, the study population was limited to mothers with stable LBW infants weighing 1500-2500 g and the findings may not be applicable to mothers with unstable or very low birthweight (<1500 g) babies. Second, information was unavailable on maternal nutrition or diet practices that might be associated with breast milk output and breastfeeding performance, infant gut function, and well-being including mental health. This information could have been useful to document adequate randomization and contextualize our findings. Third, in the studies included in the PhD thesis, the mean time to initiation of KMC in the community among intervention arm participants was 48 hours. Recently, based on findings of a large multicentric trial in five sites in Indian and African settings (152), in 2022 the WHO has recommended immediate initiation of KMC as soon as possible after birth for all preterm or LBW infants, unless critically ill, which can potentially save more lives (14). It is plausible that immediate initiation of KMC after birth along with its continued practice at home will have higher effectiveness on the maternal and child health outcomes, which may be interesting to study further. Lastly, the studies did not report long-term effect of the KMC intervention on outcomes after day-28 of birth.

The studies included in the PhD thesis were designed as efficacy trials to evaluate the effect of promotion of community-initiated KMC in LBW infants. It is possible that in an effectiveness study design i.e., in real life scenario, the effects may be lower or similar. In our efficacy trial design, the LBW infants were identified by trained workers using standardized equipment and protocols. In real-life situations in India, birth weight is measured by nurses in hospitals or by ASHA/ANM at home in case of home births, and some are missing. The

weighing equipment and protocol might vary across hospitals and communities. Therefore, in an effectiveness design, the component of identification of infants for KMC may be subjected to some degree of misclassification and may have an attenuated effect size compared with the efficacy design. To simulate feasibility towards implementing the KMC intervention within the health system, in our efficacy trial KMC was promoted using ASHA and ANM like female study workers belonging from the area with similar educational qualification as that of the government workers. In an effectiveness design, where the intervention is delivered through the existing government health system workers, it is possible that the intervention effect might be lower or similar depending on the adherence to intervention achievable.

There are several areas that require further research to improve knowledge on the full range of benefits of KMC. First, observation-based assessment of infant breastfeeding may be associated with some degree of subjectivity and bias. Hence, evaluation of the effect of KMC on the volume of infant breast milk intake using novel and accurate methods like stable-isotope technology will be relevant (153). It may be interesting to understand the effects of promotion of SSC and breastfeeding separately, but as KMC is recommended as a package it may be difficult to design such a study from an ethics perspective. Second, to clarify the association of KMC with EED, a comprehensive evaluation is needed. EED is a broad entity encompassing several aspects of gut function and systemic inflammation and is still not fully understood (77, 80, 134). Here, it will also be useful to understand if KMC practice is associated with any alteration of viral and bacterial pathogens (Enteroviruses, Adenoviruses, Campylobacter, and diarrheagenic Escherichia coli) in the infant gut. In addition, it is plausible that KMC might influence the establishment of a Bifidobacterium rich healthy gut microbiome in the LBW infants through better breastfeeding. Research to explore the effect of KMC on gut microbiota abundance and diversity may explain its clinical benefits on infant nutrition and infection related outcomes. Third, it will be important to evaluate any long-term effects of KMC on maternal mental health, including acute and chronic stress. Lastly, literature on the effect of KMC in very low birth weight (VLBW), unstable infants is very limited. Research to evaluate the effect of KMC in VLBW and unstable infants on clinical and biological outcomes would benefit infant survival programs.

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10 APPENDICES

Appendix 1. Infant Breastfeeding Assessment Tool*

OBSERVE	Score = 3	Score = 2	Score = 1	Score = 0	SCORE for each parameter
Readiness to feed (Observe what the mother did to start the baby to feed)	Placed the baby on the breast as no effort was needed.	Used mild stimulation such as patting or burping.	Needed more stimulation to start feeding e.g. rubbed baby's body or limbs vigorously at beginning and during feeding.	Could not be aroused	
Rooting (Moves mouth towards mother's nipple when stimulated) Observe how the baby responded	Rooted effectively at once.	Needed coaxing (baby talk), prompting or encouragement.	Rooted poorly even with coaxing	Did not root.	
Fixing (Latch On) Observe how long did it take from placing baby on breast to start feeding	Feeds immediately	Takes 3-10 minutes to start	Takes over 10 minutes to start	Did not start feeding	
Sucking pattern	Sucked well throughout on one or both breasts.	Sucked on & off but needed encouragement.	Sucked poorly, weak sucking; sucking efforts for short periods.	Did not suck.	
TOTAL Infant Breastfeeding assessment SCORE (A+B+C+D)					

*Reference: Matthews MK. Developing an instrument to assess infant breastfeeding behaviour in the early neonatal period. Midwifery. 1988;4(4):154-65.

Appendix 2. Patient Health Questionnaire – 9*

Over the last 2 weeks, how often have you been bothered by any of the following problems?

(Instruction: Tick the boxes appropriately as per the responses of the mothers)

Over the last 2 weeks do you have	Not at all	Several Day (1-6 days)	More than hal the days (7-1(days)	Nearly everyday (11-14 days)
Little interest or pleasure in doing things	0	1	2	3
Feeling down, depressed, or hopeless.	0	1	2	3
Trouble falling/staying asleep, sleeping too much.	0	1	2	3
Feeling tired or having little energy.	0	1	2	3
Poor appetite or overeating.	0	1	2	3
Feeling bad about yourself, or that you are a failur , or have let yourself or your family down	0	1	2	3
Trouble concentrating on things, such as reading the newspaper or watching TV	0	1	2	3
Moving or speaking so slowly that other people could have noticed OR the opposite; being so fidgety or restless that you have been moving around more than usual	0	1	2	3
Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

Total PHQ 9 score: (Total of the scores for each of the questions from A to I) = _____

*Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001;16(9):606-13.

Appendix 3. Supplementary Tables

Supplementary Table 1. Description of Baseline Variables in All Analysed Versus All Randomized Participants in Study I and III[1,2]

Variables	Study I		Study III	
	All Analysed N=528	All Randomized N=550	All Analysed N=1826	All Randomized N=1950
Household characteristics				
Wealth Quintiles				
Least poor	113 (21.4)	118 (21.4)	313 (17.1)	338 (17.3)
Less poor	111 (21.0)	113 (20.6)	338 (18.5)	363 (18.6)
Poor	102 (19.3)	106 (19.3)	382 (20.9)	402 (20.6)
Very poor	110 (20.8)	118 (21.4)	433 (23.7)	460 (23.6)
Most poor	92 (17.4)	95 (17.3)	360 (19.7)	387 (19.9)
Median number of family members (IQR)	7 (5 to 9)	7 (5 to 9)	7 (5 to 9)	7 (5 to 9)
Maternal and paternal characteristics				
Mean (SD) maternal age in years	23.3 (3.5)	22.2 (3.5)	23.3 (3.5)	23.3 (3.5)
Mean (SD) paternal age in years	26.7 (4.6)	26.6 (4.6)	26.6 (4.6)	26.5 (4.7)
Maternal education: Median (IQR) years of schooling	7 (0 to 10)	7 (0 to 10)	7 (0 to 10)	7 (0 to 10)
Birth related characteristics				
Home delivery	83 (15.7)	88 (16.0)	279 (15.3)	301 (15.4)
Birth order				
1	192 (36.4)	199 (36.3)	710 (38.9)	754 (38.6)
2 to 4	287 (54.5)	302 (55.0)	965 (52.9)	1038 (53.3)
≥5	48 (9.1)	48 (8.7)	150 (8.2)	157 (8.1)

Variables	Study I		Study III	
	All Analysed N=528	All Randomized N=550	All Analysed N=1826	All Randomized N=1950
Infant characteristics				
Sex of the baby: Female	288 (54.5)	298 (54.2)	1027 (56.2)	1090 (55.9)
Mean (SD) weight at enrolment in gm	2085 (160)	2086 (160)	2087 (159)	2084 (162)
Mean (SD) gestational age in weeks[2]	35.7 (2.1)	35.7 (2.1)	35.8 (2.0)	35.8 (2.0)
Proportion born preterm (<37 weeks)	342 (64.7)	356 (64.7)	1147 (62.8)	1217 (62.4)

[1]We have presented baseline characteristics of all analysed participants in the manuscript. This is because the primary purpose of Table 1 was to be used to screen for baseline prognostic variables that were unequally distributed between the intervention and control arm (a priori defined as a relative difference of >10%). In Study I & III, the loss to follow-up was low (<7%) and we did not find any major differences in the distribution of baseline characteristics in all randomized against all analysed participants.

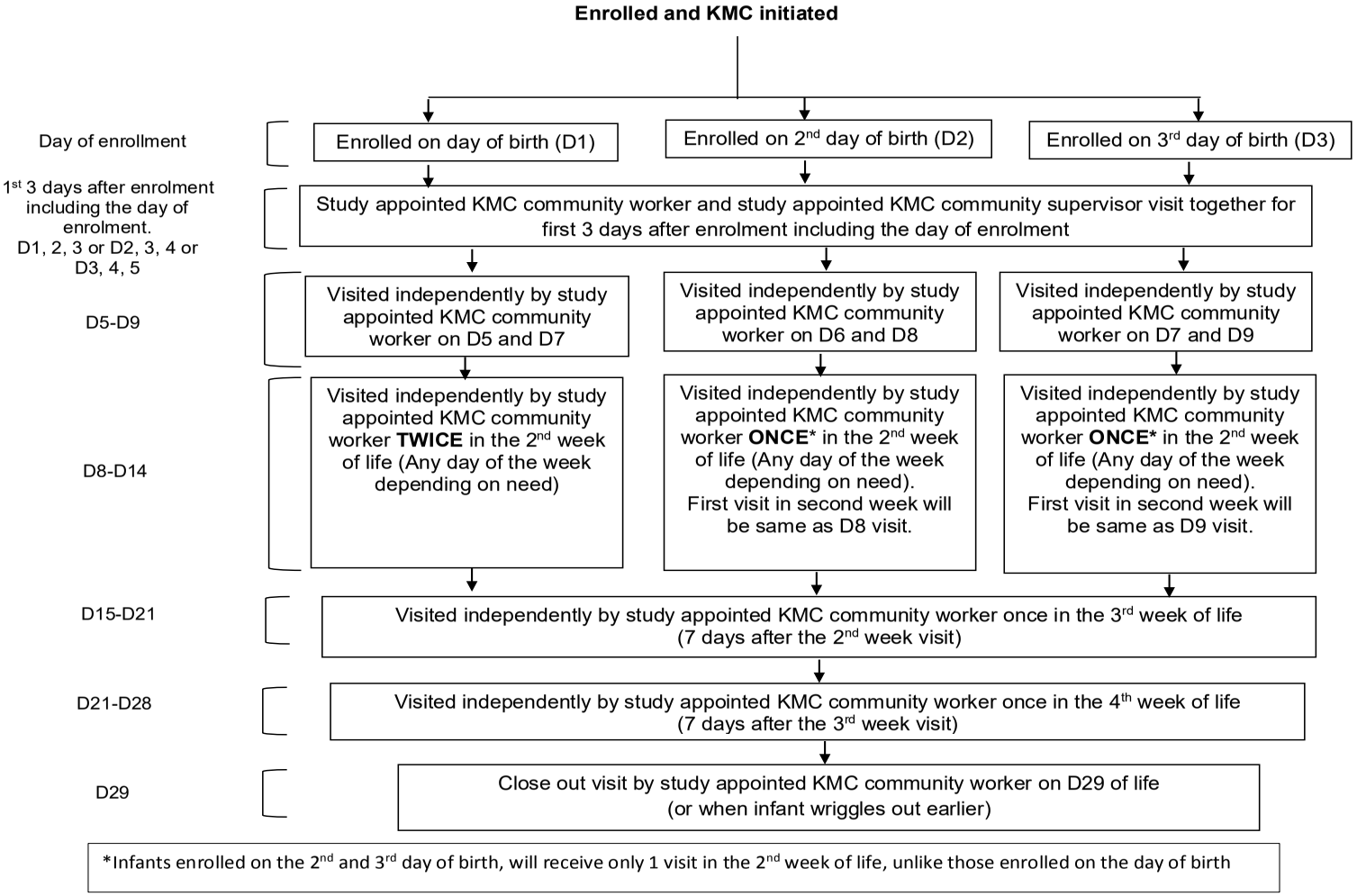
[2]The loss to follow up in Study II was negligible

Supplementary Table 2. Description of Baseline Variables in the PhD Studies and the Primary KMC Trial

Variables	Study I		Study II		Study III		Primary KMC trial	
	Control arm (N=276) n (%)	KMC arm (N=252) n (%)	Control arm (n=100) n (%)	KMC arm (n=100) n (%)	Control arm (N=852) n (%)	KMC arm (N=974) n (%)	Control arm (N=3922) n (%)	KMC arm (N=4480) n (%)
Household characteristics								
Wealth Quintiles								
Least poor	53 (19.2)	60 (23.8)	19 (19.0)	16 (16.0)	146 (17.1)	167 (17.2)	794 (20.2)	891 (19.9)
Less poor	61 (22.1)	50 (19.8)	25 (25.0)	27 (27.0)	157 (18.4)	181 (18.6)	766 (19.5)	917 (20.5)
Poor	53 (19.2)	49 (19.4)	17 (17.0)	26 (26.0)	178 (20.9)	204 (20.9)	783 (20.0)	891 (19.9)
Very poor	58 (21.0)	52 (20.6)	25 (25.0)	13 (13.0)	204 (23.9)	229 (23.5)	768 (19.6)	904 (20.2)
Most poor	51 (18.5)	41 (16.3)	14 (14.0)	18 (18.0)	167 (19.6)	193 (19.8)	811 (20.7)	871 (19.5)
Maternal characteristics								
Mean (SD) maternal age in years	23.4 (3.7)	23.2 (3.3)	24.1 (3.7)	23.1 (3.2)	23.1 (3.5)	23.4 (3.6)	23.4 (3.8)	23.3 (3.7)
Maternal education: Median (IQR) years of schooling	7 (0 to 10)	7 (0 to 10)	8 (0 to 12)	6 (0 to 10)	7 (0 to 10)	7 (0 to 10)	6.2 (5.2)	6.0 (5.2)
Birth related characteristics								
Home delivery	47 (17.0)	36 (14.3)	14 (14.0)	16 (16.0)	129 (15.1)	150 (15.4)	737 (18.8)	828 (18.5)
Birth order								
1	96 (34.9)	96 (38.1)	35 (35.0)	37 (37.0)	343 (40.3)	367 (37.7)	1543 (39.3)	1728 (38.6)
2 to 4	151 (54.9)	136 (54.0)	56 (56.0)	56 (56.0)	450 (52.8)	515 (52.9)	2077 (53.0)	2361 (52.8)
≥5	28 (10.2)	20 (7.9)	9 (9.0)	7 (7.0)	59 (6.9)	91 (9.4)	302 (7.7)	386 (8.6)
Infant characteristics								
Sex of the baby: Female	147 (53.3)	141 (55.9)	52 (52.0)	59 (59.0)	470 (55.2)	557 (57.2)	2181 (55.6)	2573 (57.4)
Mean (SD) weight at enrolment in gm	2076 (166)	2095 (154)	2095 (162)	2086 (139)	2085 (159)	2089 (160)	2073 (166)	2073 (168)
Mean (SD) gestational age in weeks[1]	35.6 (2.2)	35.7 (2.0)	35.9 (1.9)	35.9 (1.6)	35.8 (2.0)	35.8 (2.0)	35.7 (2.0)	35.7 (2.0)
Proportion preterm (<37 weeks)	178 (64.9)	164 (65.1)	62 (62.0)	63 (63.0)	535 (62.8)	612 (62.8)	2512 (64.0)	2893 (64.6)

[1]Note in the publication of the Primary KMC trial (Lancet 2019), gestational age was estimated based on last menstrual period as reported by mothers. But here we have considered gestational age as per the ultrasonography (USG) reports, when available. If USG was not available, the gestational age was estimated based on last menstrual period as documented in hospital records or as per maternal recall, whichever was available, in the given order of preference.

Appendix 4. Visit Schedule for Intervention Delivery Based on Day of Enrollment



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Effect of kangaroo mother care in low birth weight infants on breastfeeding performance, gut function, and maternal depressive symptoms in low middle income populations in the Indian subcontinent

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11 ORIGINAL PUBLICATIONS

PUBLICATION I

Effect of community-initiated kangaroo mother care on breastfeeding performance in low birthweight infants: A randomized clinical trial

Bireshwar Sinha, Halvor Sommerfelt, Per Ashorn, Sarmila Mazumder, Sunita Taneja, Rajiv Bahl, Nita Bhandari

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ORIGINAL ARTICLE

Maternal & Child Nutrition WILEY

Effect of community-initiated kangaroo mother care on breastfeeding performance in low birthweight infants: A randomized clinical trial

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Abstract

This individually randomized trial was conducted to estimate the effect of promoting community-initiated kangaroo mother care (ciKMC) in low birthweight (LBW) infants on infant breastfeeding performance. It was designed as a substudy within a larger primary trial on ciKMC and infant survival. Five hundred fifty stable LBW mother-infant dyads (1500–2250 g) who provided consent, were consecutively enroled for breastfeeding performance assessment. The ciKMC intervention included promotion and support of continuous skin-to-skin contact and exclusive breastfeeding (EBF) through home visits during the neonatal period. The primary outcome was effective breastfeeding performance indicated by an infant breastfeeding assessment tool score of ≥10 after the end of the neonatal period. As secondary outcomes, we reported maternal satisfaction related to infant breastfeeding, and EBF after the end of the neonatal period. We completed outcome assessments in 96% of participants. In the ciKMC arm, 92% of the infants showed effective breastfeeding performance against 81% in the control arm [adjusted prevalence ratio (aPR): 1.24, 95% confidence interval (CI): 1.16–1.32]. In the ciKMC arm, 65% of the mothers reported to be very satisfied with their infants' breastfeeding against 51% in the control arm (aPR: 1.22, 95% CI: 1.05–1.41). The proportion of infants practicing EBF was 89% in the ciKMC arm against 45% in the control arm (aPR: 1.62, 95% CI: 1.45–1.81). Our study findings suggest that promotion of ciKMC can improve effective breastfeeding, EBF and maternal satisfaction related to breastfeeding in LBW infants.

KEYWORDS
breastfeeding performance, infant, Kangaroo mother care, low birthweight

1 | INTRODUCTION

The World Health Organization (WHO) recommends initiation of breastfeeding within 1 h of birth and exclusive breastfeeding (EBF) for the first 6 months of life for all infants including those born low birthweight (LBW) <2500 g (WHO, 2011, 2021). A meta-analysis indicates that, compared to infants exclusively breastfed during the first 6 months of life, all-cause mortality is 14 times higher in non-breastfed infants and 5 times higher in partially breastfed infants (Sankar et al., 2015). In addition, breastfeeding is associated with reduced risk of breast cancer, ovarian cancer, and diabetes in the mothers (Victora et al., 2016). Mothers of infants born preterm, that is, before 37 completed weeks of gestation or LBW, often report perceived breastmilk insufficiency, making practice and continuation of EBF difficult (Edmond & Bahl, 2006; Maastrup et al., 2014; Mathur & Dhingra, 2009; Sethi et al., 2017). A multicountry cohort study reported that the practice of non-EBF at 42 days of age was ~30% higher in Indian LBW infants than those with birthweight ≥2500 g (Patel et al., 2015). In a study in India among late preterm infants (34–36 weeks of gestation), EBF practice at the end of the first postnatal week after birth was reported to be 33%, with a feeling of insufficient milk as the most common reason for non-EBF practice (Harsha & Kumar, 2017).

Achieving a high prevalence of EBF in the LBW or preterm infants can be challenging. Poor breastfeeding in these infants is associated with attachment or latching-on difficulties, drowsiness, poor and intermittent sucking, poor coordination of sucking and swallowing and disorganized feeding behaviour (Dosani et al., 2016; Harsha & Kumar, 2017). Moreover, poor breastfeeding behaviour can also be associated with parental stress and perceived breastfeeding insufficiency in the mother (Dongre et al., 2010; Dosani et al., 2016). The WHO and the Government of India recommend Kangaroo mother care (KMC), an intervention encompassing skin-to-skin contact (SSC) and EBF, to improve survival in LBW babies (Conde-Agudelo & Diaz-Rossello, 2016; GOI, 2014; WHO, 2003). SSC following birth may promote early and improved attachment of the infant to the mother's breast, leading to successful breastfeeding (Dewey et al., 2003; Winberg, 2005). Our randomized controlled trial in India among 8402 LBW infants demonstrated that promotion of community-initiated KMC (ciKMC) improves post-enrolment neonatal survival by 30% (Mazumder et al., 2019). It is plausible that ciKMC promotion may improve breastfeeding performance in LBW infants, but high-quality evidence from randomized trials is lacking.

Our primary objective was to test the hypothesis that promotion of ciKMC improves effective breastfeeding performance (Matthews, 1988) in LBW infants after the end of the neonatal period that is, 28 days after birth. As secondary objectives, we estimated the effect of promotion of ciKMC in LBW infants on the maternal perception of infant breastfeeding and the EBF prevalence after the end of the neonatal period.

Key messages

- In low birthweight (LBW) infants, breastfeeding can be challenging with low prevalence of exclusive breastfeeding (EBF).
- Our trial in North India among 550 LBW infants showed that promotion of community-initiated Kangaroo mother care (ciKMC) can substantially improve infant breastfeeding performance.
- ciKMC promotion substantially improved EBF prevalence at the end of the neonatal period, number of breastfeeds per day and duration of each breastfeed. It also enhanced mother's satisfaction with their infant's breastfeeding performance.

2 | METHODOLOGY

2.1 | Study design and participants

This individually randomized parallel arm trial was developed as a substudy within a larger primary trial (ClinicalTrials.gov NCT02653534) (Mazumder et al., 2017) specifically to assess infant breastfeeding performance. The primary trial was conducted in rural and semiurban low-income populations of Faridabad and Palwal districts in Haryana, India. Newborns weighing 1500–2250 g and their mothers were screened for enrolment as early as possible, within 72 h of birth. Infants were excluded if KMC had already been initiated in a birth facility, not weighed within 72 h of birth, or they were unable to feed, had breathing problems, gross congenital malformations, reported less than normal movements (WHO, 2014) or mothers were not living with their babies or intending to move away over the next 6 months. Additionally, in this substudy, we excluded twins, triplets and infants in whom breastfeeding was not initiated. In the primary trial enrolments were done between July 2015 and October 2018. For infant breastfeeding performance assessment, we enroled consecutive eligible and consenting mother-infant dyads from April 2017 onwards (Figure 1) in the substudy using the randomization sequence of the primary trial.

The randomization list was prepared by an off-site statistician using random permuted blocks of variable size. Allocation of participant identification number was conducted by an independent coordinator using serially numbered opaque sealed envelopes (Mazumder et al., 2017, 2019).

2.2 | Intervention and usual care

The ciKMC intervention comprised of promotion and support of continuous and prolonged SSC and EBF. The intervention delivery team visited homes of the infant-mother dyads allocated to the

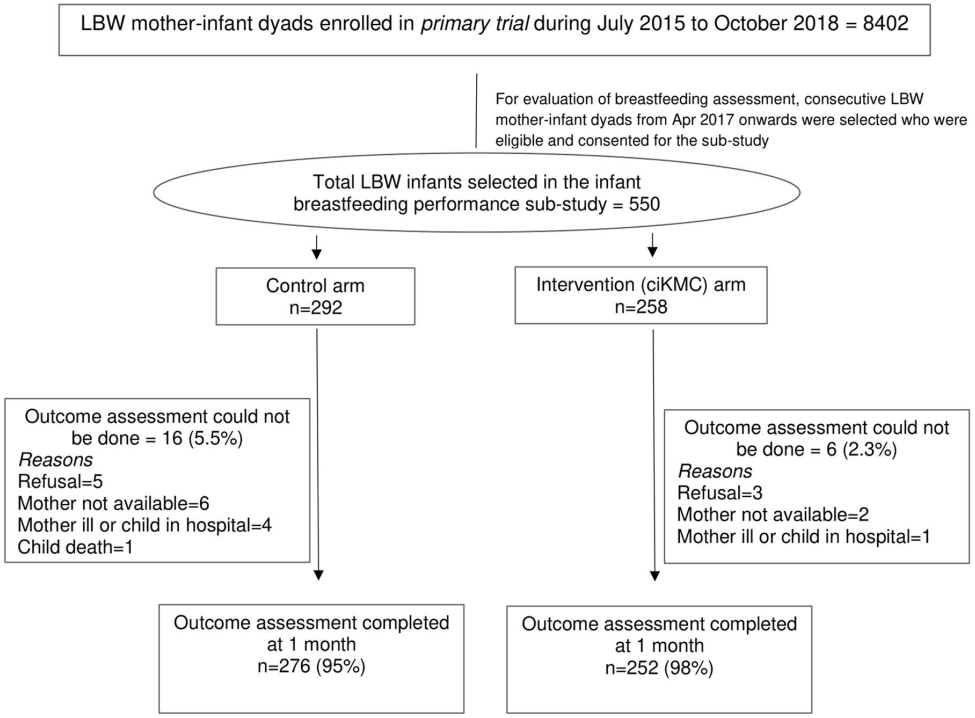

FIGURE 1 Participant flow in the trial. ciKMC, community-initiated kangaroo mother care; LBW, low birthweight.

ciKMC arm to initiate and support KMC on Days 1, 2, 3, 5, 7, 10, 14, 21 and 28 after birth to observe and solve any problems related to SSC or breastfeeding. Mothers were counselled to practice SSC for as long as possible during day and night, with the assistance of other family members. Visits continued till 28 days of age or until the baby wriggled out of the KMC position and no longer accepted SSC, if earlier. Referral of ill infants in both ciKMC and control arms was facilitated through government Accredited Social Health Activists (ASHAs) (NHM, 2014). All infants in both the ciKMC and control arms received home-based postnatal care visits by ASHAs as implemented through the health system (MOHFW, 2014). Further details of the intervention delivery have been published previously (Mazumder et al., 2019).

2.3 | Study outcomes and its assessment

Our primary outcome for the substudy was effective breastfeeding performance after the end of the neonatal period, indicated by a score of ≥10 in the infant breastfeeding assessment tool (IBFAT) (Khadivzadeh & Karimi, 2009; Matthews, 1988; Moore & Anderson, 2007). In addition, we report median IBFAT score, and the proportion of infants showing the score's individual components, that is, effective readiness to feed, rooting, fixing and sucking pattern. Each of these components are scored from 0 to 3 on a Likert scale, '0' indicating the worst and '3' indicating effective performance, with the total score ranging from 0 to 12 (Supporting Information: Appendix 1). IBFAT was developed by Matthews M. K. in 1988 (Matthews, 1988) has been found to be a reliable and validated tool for assessment of infant breastfeeding performance with high correlation ($R > 0.7$) with other available instruments like LATCH and the Mother Baby Assessment tool (Altuntas et al., 2014). It has been used in several previous trials (Moore & Anderson, 2007; Srivastava et al., 2014).

As secondary outcomes we assessed maternal satisfaction related to infant breastfeeding (based on 7 day recall) and EBF (24 h recall) at the end of the neonatal period (WHO & UNICEF, 2021). For assessment of maternal satisfaction related to infant breastfeeding, the mother was asked about her perception related to breastfeeding of the infant in the last 7 days if she was 'very satisfied', 'satisfied', 'somewhat satisfied' or 'not satisfied' with the way the baby fed during each feeding. This four-point Likert scale was originally used by Matthews (1988) and thereafter adapted and has been used previously in different settings including in India (Matthews, 1988; Srivastava et al., 2014).

Outcome assessment specific to the substudy was conducted by a trained team of four workers who had at least graduate-level education, hereby referred as 'substudy outcome assessment team'. This team was not involved in intervention delivery or ascertainment of any other outcomes, including the duration of

SSC, which was done by the primary trial outcome assessment workers. Training of the substudy outcome assessment team in breastfeeding assessment was conducted by a trainer certified in infant and young feeding counselling by the Breastfeeding Promotion Network of India. After training, a standardization exercise was done in which the IBFAT questionnaire scores assigned by the substudy outcome assessment team workers were matched with those of the certified trainer. An interclass correlation coefficient of >0.75 (Koo & Li, 2016) between the workers and the trainer for all the four components of the tool was a criterion to allow the workers to conduct the IBFAT assessment. All outcomes were assessed in the homes of the participants.

2.4 | Statistical analysis

The sample size for the substudy was calculated prior to its initiation. With the assumption that 50% of the infants in the control arm would show effective breastfeeding (Kishore et al., 2009) and to be able to detect a minimum of 25% relative improvement in the in this proportion with 80% power, 95% confidence interval (CI) and 10% attrition, we enroled a total of 550 participants.

Analyses were conducted on an intent-to-treat basis using STATA version 16 (Stata Corporation). As outcome data were available in >95% of the study participants, we did not impute for missing data (Jakobsen et al., 2017).

We estimated prevalence ratios (PRs) for effective breastfeeding performance between the study arms using generalized linear models (GLM) of the binomial family with a log-link. In addition, we estimated absolute risk difference and the number needed to treat. Maternal age, birth order, place of delivery, sex of the baby, weight at enrolment and preterm birth were considered potential confounding factors based on prior knowledge if associated with the primary outcome at $p < 0.1$ in univariable analysis. To estimate the adjusted prevalence ratio (aPR), we included a potential confounding factor if it were unequally distributed between the study arms at baseline (a priori defined as a relative difference of >10%) and the baseline IBFAT scores in the multivariable GLM analysis. Design effects of infants enroled from a single household were accounted for using Stata's robust variance estimator (cluster) option. We used the same analytical approaches for the other categorical study outcomes that is, maternal satisfaction related to infant breastfeeding (very satisfied), and EBF.

We conducted subgroup analyses, decided a priori, to estimate whether the effect of ciKMC on effective breastfeeding performance was different in preterm infants (<37 weeks gestation) compared with full-term infants (≥37 weeks gestation). To quantify any biological interaction between ciKMC promotion and preterm birth, we estimated the absolute excess risk due to interaction using interaction term in the GLM analysis. Gestational age was estimated from the ultrasonography reports, when available, or based on the last menstrual period as documented in hospital records or as per maternal recall, in the given order of preference.

For IBFAT scores, we reported the median and interquartile range, given its asymmetric left-skewed distribution. We used the Wilcoxon rank-sum test to evaluate the hypothesis that promotion of ciKMC was associated with higher IBFAT scores. To explore the consistency of our analysis across different cut-offs of IBFAT score, we plotted the cumulative density frequency of the total IBFAT score after the end of the neonatal period across study arms.

3 | RESULTS

The baseline characteristics were similar in the two study arms other than for home delivery, and birth order (≥5), where the relative differences in proportions between study arms exceeded 10% (Table 1). Among the 550 enroled participants, we completed outcome assessments in 98% (252/258) of the infants in the ciKMC arm and 95% (276/292) of the infants in the control arm (Table 2).

All mothers in the ciKMC arm practiced SSC, while 8.7% (24/276) of the mothers in the control arm reported SSC practice. In the ciKMC arm, the median (interquartile range [IQR]) age of the infant at ciKMC initiation was 48 (19–72 h); the mean (SD) duration of SSC practice was 28 (2.4) days with 12 (3.8) hours per day. In the control arm, the mean (SD) duration of SSC practice was 2 (7.1) days with 1 (2.1) hour per day.

The median (IQR) IBFAT score at enrolment was 11 (9–12) both in the ciKMC arm and in the control arm infants. At the end of the neonatal period (mean age 28 days in both ciKMC and control arms), 92% (232/252) of the infants in the ciKMC and 81% (223/276) in the control arm showed effective breastfeeding performance. The aPR (95% CI) for effective breastfeeding performance adjusted for potential confounding baseline factors (place of delivery and birth order) and baseline IBFAT score was 1.24 (1.16–1.32), corresponding to an effect of 24% (16%–32%); the unadjusted PR was 1.14 (1.06–1.22). The absolute unadjusted risk difference (95% CI) for effective breastfeeding performance was 11.2% (5.5%–16.9%) corresponding to a number needed to treat of 9 infants (6–18).

At the end of the neonatal period, the median (IQR) of IBFAT score was 12 (12–12) in the ciKMC arm and 12 (10–12) in the control arm infants ($p < 0.001$). The cumulative frequency plot showed a right shift of the IBFAT score in the ciKMC arm compared with the control arm (Figure 2), showing that at any given cut-off of the IBFAT score, a higher proportion of infants in the ciKMC arm was above the cut-off score (Supporting Information: Table 1).

A higher proportion of infants in the ciKMC arm than the control arm showed effective readiness to feed (88% vs. 80%; aPR: 1.13, 95% CI: 1.06–1.21), effective rooting (91% vs. 83%; aPR: 1.18, 95% CI: 1.12–1.25), effective fixing (91% vs. 73%; aPR: 1.30, 95% CI 1.21–1.40) and effective sucking pattern (87% vs. 67%; aPR: 1.32, 95% CI: 1.21–1.45) (Supporting Information: Table 2).

In the subgroup of babies born preterm, the aPR (95% CI) of effective breastfeeding performance in the ciKMC arm was 1.30 (1.18–1.42) versus 1.13 (1.07–1.19) among full-term infants. Absolute excess risk due to interaction between preterm birth and ciKMC for the primary outcome was estimated to be 0.05 (95% CI: −0.05 to 0.16).

TABLE 1 Baseline characteristics of participants in the control and intervention arm[a]

Variables	Control (*N* = 276) *n* (%)	ciKMC (*N* = 252) *n* (%)
Household characteristics		
Wealth quintiles		
Least poor	53 (19.2)	60 (23.8)
Less poor	61 (22.1)	50 (19.8)
Poor	53 (19.2)	49 (19.4)
Very poor	58 (21.0)	52 (20.6)
Most poor	51 (18.5)	41 (16.3)
Religion: Hindu	225 (81.8)	204 (80.9)
Median number of family members (IQR)	7 (5–9)	7 (5–10)
Maternal and paternal characteristics		
Mean (SD) maternal age in years	23.4 (3.7)	23.2 (3.3)
Mean (SD) paternal age in years	26.9 (4.6)	26.4 (4.6)
Maternal education: Median (IQR) years of schooling	7 (0–10)	7 (0–10)
Birth related characteristics		
Home delivery	47 (17.0)	36 (14.3)
Birth order		
1	96 (34.9)	96 (38.1)
2–4	151 (54.9)	136 (54.0)
≥5	28 (10.2)	20 (7.9)
Infant characteristics		
Sex of the baby: Female	147 (53.3)	141 (55.9)
Mean (SD) weight at enrolment in gram	2076 (166)	2095 (154)
Mean (SD) gestational age in weeks[b]	35.6 (2.2)	35.7 (2.0)
Proportion born preterm (<37 weeks)	178 (64.9)	164 (65.1)

Abbreviation: ciKMC, community-initiated kangaroo mother care.

[a]65% (360/550) had an ultrasound for gestational age assessment.

[b]Data presented are number/percentages unless indicated otherwise.

TABLE 2 Effect of community-initiated kangaroo mother care on effective infant breastfeeding at the end of neonatal period

Outcome variable	Population	Control *n/N* (%)	ciKMC *n/N* (%)	Unadjusted PR (95% CI)	Adjusted[a] PR (95% CI)
Effective infant breastfeeding (IBFAT score ≥10)	All infants	223/276 (80.8)	232/252 (92.1)	1.14 (1.06–1.22)	1.24 (1.16–1.32)
	Preterm infants	136/178 (76.4)	147/164 (89.6)	1.17 (1.06–1.29)	1.30 (1.18–1.42)
	Term infants	87/98 (88.8)	85/88 (96.6)	1.08 (1.00–1.18)	1.13 (1.07–1.19)

Abbreviations: CI, confidence interval; ciKMC, community-initiated kangaroo mother care; IBFAT, infant breastfeeding assessment tool; IQR, interquartile range; PR, prevalence ratio.

[a]Adjusted for place of delivery, birth order and baseline IBFAT score. The design effect of more than one infant being included from a single household was accounted for by using Stata's cluster option to obtain a robust variance estimator.

In the ciKMC arm, 65% (164/252) of the mothers reported to be very satisfied with their infant's breastfeeding at the end of the neonatal period. In the control arm, the corresponding proportion was 51% (141/276, aPR: 1.22, 95% CI: 1.05–1.41, Table 3). Breast or nipple problems at the end of the neonatal period was reported by 5.1% (13/252) of the mothers in the ciKMC arm and 8.7% (24/276) of the mothers in the control arm (PR: 0.58, 95% CI: 0.30–1.11).

The mean interval between birth and breastfeeding initiation was 4.4 (9.8) h in the ciKMC arm infants and 4.7 (10.5) h in the control arm infants. The proportion of infants practicing EBF at the end of the neonatal period was 89% (225/252) in the ciKMC arm and 45% (123/276) in the control arm (aPR: 1.62, 95% CI: 1.45–1.81, Table 3). The reported median (IQR) number of breastfeeds per day (24 h recall) was 12 (12–14) in the ciKMC arm and 11 (9–14) in the control arm ($p < 0.001$). The reported mean (SD) duration of each breastfeed was 15.5 (5.1) min in the ciKMC arm and 10.1 (5.1) min in the control arm infants (mean difference: 5.4, 95% CI: 4.5–6.3).

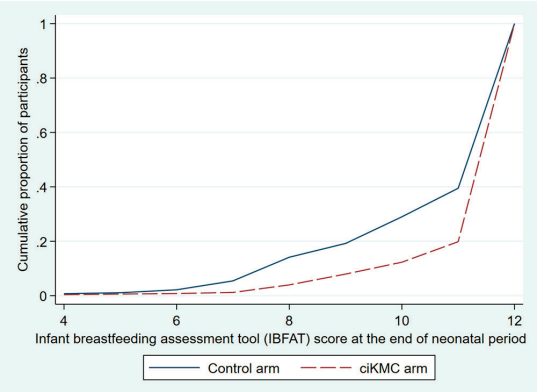

FIGURE 2 Cumulative frequency plot showing IBFAT[1] scores at the end of neonatal period. ciKMC, community-initiated kangaroo mother care; IBFAT, infant breastfeeding assessment tool.

4 | DISCUSSION

We aimed to estimate the effect of ciKMC promotion among LBW infants on effective breastfeeding performance, maternal satisfaction related to infant breastfeeding and EBF at the end of the neonatal period. In a sample of 550 stable LBW infants included in our trial, the prevalence of effective breastfeeding performance was substantially higher in the ciKMC arm than in the control arm. Our findings indicate that promoting ciKMC for 9 LBW infants would result in 1 more infant breastfeeding effectively. The ciKMC infants showed improved performance in all four components of the IBFAT, that is, readiness to feed, rooting, fixing and sucking pattern. ciKMC promotion also enhanced the mothers' satisfaction with their infant's breastfeeding performance. EBF at the end of the neonatal period, reported number of breastfeeds per day, and the duration of each breastfeed was substantially higher among ciKMC arm infants compared to among the control arm infants.

In our study, selection bias is unlikely, because of effective randomization, allocation concealment and low attrition. To minimize misclassification bias, we conducted a standardization exercise prior to the IBFAT assessment. Some of the outcome measurements were based on mothers recall like mother's perception of infant breastfeeding (7-day recall) and EBF (24 h recall). The short recall periods minimize the possibility of recall bias. To minimize observer's bias, IBFAT assessment at the end of the intervention period was done by an independent trained team, unaware of trial arm allocation and not involved in intervention delivery. However, we cannot rule out that the unmasked nature of the trial might make it possible to guess the trial arm allocation with a resultant small observer bias in a few cases.

TABLE 3 Effect of community-initiated kangaroo mother care on mother's reported perception on infant breastfeeding and breastfeeding prevalence (24 h recall) at the end of neonatal period

Outcomes	Control *N* = 276 *n* (%)	ciKMC *N* = 252 *n* (%)	Unadjusted PR (95% CI)	Adjusted PR (95% CI)
Mother's reported perception on infant breastfeeding (7-day recall)				
Very satisfied	141 (51.1)	164 (65.1)	1.28 (1.10–1.48)	1.22 (1.05–1.41)[a]
Satisfied	110 (39.8)	83 (32.9)	Reference[b]	Reference[b]
Somewhat satisfied	16 (5.8)	4 (1.6)		
Not satisfied	9 (3.3)	1 (0.4)		
Breastfeeding rates (24 h recall)				
Exclusive	123 (44.6)	225 (89.3)	1.61 (1.43–1.80)	1.62 (1.45–1.81)[c]
Predominant	81 (29.4)	22 (8.7)	Reference[d]	Reference[d]
Partial	42 (15.2)	5 (1.9)		
No	0 (0.0)	0 (0.0)		

Abbreviations: CI, confidence interval; ciKMC, community-initiated kangaroo mother care; PR, prevalence ratio.

[a]Adjusted for place of delivery and birth order and mother's reported perception on breastfeeding at baseline.

[b]For calculation of the prevalence ratio for 'very satisfied', all other categories are clubbed as the reference category.

[c]Adjusted for place of delivery and birth order.

[d]For calculation of the prevalence ratio for exclusive breastfeeding, all other categories are considered as nonexclusive breastfeeding.

Given the overall low risk of bias, we believe that our findings are internally valid. We therefore believe that the promotion of ciKMC, in low resource settings such as ours, can substantially improve breastfeeding performance, maternal satisfaction with breastfeeding and EBF prevalence in stable LBW infants.

Previous studies have used IBFAT to examine the effect of SSC practice in healthy or term infants in hospital settings on breastfeeding. A randomized trial in North India among 298 healthy mother-infant dyads reported that IBFAT score at 6 weeks was increased in infants who were supported to practice SSC for at least 2 h. Likewise, a randomized trial in Nashville, Tennessee, among full-term infants, reported a substantially higher IBFAT score in the SSC arm infants compared to controls (Moore & Anderson, 2007). Similarly, a meta-analysis including three randomized trials conducted in India, Italy and the USA, reported higher IBFAT scores in healthy infants practicing SSC compared to those with routine care in hospital settings (Ghojazadeh et al., 2019). Another study in Iran showed that full-term infants randomized to practice SSC for 1 h after birth had higher effective readiness to feed, effective sucking, effective latching and effective rooting (Beiranvand et al., 2014). Our trial findings substantiate the observations from such studies and provide rigorous evidence that promotion of community initiated KMC in the population of stable LBW infants can substantially improve breast-feeding performance.

Studies have also reported the effect of SSC or KMC on infant breastfeeding rates and maternal satisfaction. A previous meta-analysis reported that practice of SSC or KMC initiated in hospitals in stable low birthweight infants is associated with higher EBF rates during the first 6 months of life and longer duration of breastfeeding (Conde-Agudelo & Diaz-Rossello, 2016; Moore et al., 2016). An earlier study in North India reported that the scores of mothers' perception about their infant's breastfeeding, as measured on a four-point Likert scale, were substantially higher in the SSC arm compared to controls (Srivastava et al., 2014). Our findings concur with these findings and suggest that KMC initiated in the community or can improve EBF prevalence and maternal satisfaction with breastfeeding.

Biologically, the concept of SSC evolved from animal studies which suggested that maintenance of the maternal milieu after birth of small or premature babies may help to promote innate behaviours in the newborn and the mother, leading to successful breastfeeding and increased survival. Closeness with the mother is associated with regulation of different aspects of neonatal physiology, including behavioural, cardiorespiratory, digestive and hormonal systems (Hofer, 2006; Moore et al., 2016). Small-born infants, that is, those who are LBW or preterm, are often separated from their mother after birth, which may affect development of these physiological and behavioural systems. The effect of ciKMC on effective infant breastfeeding as well as improved maternal satisfaction is plausible because of improved feedback mechanisms between the mother and the baby, better mother-infant bonding, reduced stress and improved breastfeeding confidence (Lau, 2018). The lower proportion of reported breast or nipple related problems in ciKMC arm mothers may indicate a maternal benefit, although our estimate for that effect was statistically imprecise. The observed tendency towards a higher PR of effective breastfeeding in preterm than in term infants may be relevant given that the former group of infants are at a higher risk of non-EBF (Ayton et al., 2012). Nonetheless, we acknowledge the limitations of this subgroup analysis in that we had not stratified the randomization based on preterm or term births and the statistical precision of the absolute excess risk due to interaction was low.

Despite the low likelihood of bias and error in our trial, there were some limitations. Our study was limited to the population of stable LBW infants weighing 1500–2250 g in a low to middle income setting in India. The findings may not be generalizable to unstable or very low birthweight infants <1500 g or in different settings. For assessment of infant breastfeeding performance, we used the IBFAT which has been widely used for this purpose but is not a standard acceptable tool in all settings. Observation-based assessment of infant breastfeeding is associated with some degree of subjectivity and therefore estimation of volume of breast milk intake with newer methods like stable-isotope technology may add value. For assess-ment of breastfeeding performance, several other tools are available (Altuntas et al., 2014; Ingram et al., 2015), and the development of a standard tool to assess infant breastfeeding performance to allow comparisons across studies and settings will be important in future.

5 | CONCLUSION

Our study findings support the promotion of ciKMC as an intervention for LBW babies to improve effective breastfeeding, EBF and maternal satisfaction related to breastfeeding. Given the benefits, integration of ciKMC within the essential newborn care programs in low-middle income settings should be encouraged.

AUTHOR CONTRIBUTIONS

Bireshwar Sinha and Halvor Sommerfelt had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Concept and design*: Bireshwar Sinha and Halvor Sommerfelt. *Acquisition, analysis or interpretation of data*: Bireshwar Sinha, Halvor Sommerfelt, Per Ashorn, Sarmila Mazumder and Sunita Taneja. *Drafting of the manuscript*: Bireshwar Sinha, Halvor Sommerfelt and Per Ashorn. *Critical revision of the manuscript for important intellectual content*: All authors. *Statistical analysis*: Bireshwar Sinha, Halvor Sommerfelt and Per Ashorn. *Obtained funding*: Bireshwar Sinha, Nita Bhandari and Halvor Sommerfelt. *Supervision*: Halvor Sommerfelt, Ashorn, Per Bahl and Nita Bhandari.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The primary custodian of the data is Centre for Health Research and Development, Society for Applied Studies (CHRD SAS), India. As per the Institutional policy deidentified data will be made available on request for the purpose of checking consistency or supporting the analyses presented in this scientific manuscript.

ETHICS STATEMENT

Ethics approval was obtained from the Institutional Ethics Review Committee and the Regional Committee for Medical and Health Research Ethics (REK) in Western Norway. The substudy was separately registered with Clinical trials registry-India (CTRI/2017/04/008430). Written informed consent was obtained from the mothers of the eligible infants before enrolment. The participants were identified by study numbers to assure confidentiality and anonymity. The study is reported as per the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline (Appendix 2) (Schulz et al., 2010).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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PUBLICATION II

Effect of Community-Initiated Kangaroo Mother Care on Fecal Biomarkers of Gut Function in Low Birth Weight Infants in North India: A Randomized Clinical Trial

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Title: Effect of community-initiated kangaroo mother care on fecal biomarkers of gut function in low birthweight infants in North India: a randomized clinical trial

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ABSTRACT (Unstructured)

This individually randomized trial was conducted to estimate the effect of promoting community-initiated kangaroo mother care (ciKMC) in low birthweight (LBW) infants on gut inflammation and permeability. Participants included 200 stable LBW infants (weighing 1500-2250 g) in North India enrolled between May to October 2017. The ciKMC intervention included promotion and support of continuous skin-to-skin contact and exclusive breastfeeding through home visits. The mothers in the intervention arm were supported to practice ciKMC until 28 days after birth, i.e. the neonatal period, or till the baby wriggled out of KMC position, if earlier. Infant stool specimens were collected during the first week of birth, and within one week after end of the neonatal period. Concentrations of fecal neopterin (nmol/L), myeloperoxidase (ng/mL), and alpha-1-antitrypsin (μg/mL) were determined using ELISA, and composite enteric enteropathy (EE) score at end of the neonatal period was calculated by principal component analysis. We did not find any substantial difference in means between the ciKMC and control arm infants in the log-transformed values of neopterin (0.03; 95% CI -0.15 to 0.21), myeloperoxidase (0.28; 95% CI -0.05 to 0.61) and alpha-1-antitrypsin (0.02; 95% CI -0.30 to 0.34). The mean (SD) composite EE score was 13.6 (7.5) in the ciKMC and 12.4 (8.3) in the control arm infants, and the adjusted difference in means was negligible, 0.4 (95% CI -1.8 to 2.7). Our findings suggest that the promotion of ciKMC did not affect gut inflammation and permeability in our target population of low birthweight infants in North India.

Key words: Kangaroo mother care, Enteropathy, Gut inflammation, Gut permeability, low birth weight, infant

INTRODUCTION

Gut function among young children in low-income communities is postulated to be one of the important drivers of poor growth. Environmental enteric dysfunction (EED) is described as a broad syndrome with various alterations in gut function including increased gut inflammation, altered gut permeability, villous blunting, and crypt hyperplasia as a consequence of chronic exposure to enteropathogens.[1] EED leads to a vicious cycle of reduced intestinal absorptive capacity, which in turn causes protein-energy and micronutrient malnutrition, and thereby poor growth.[1, 2, 3] A multicentric cohort study in 8 countries showed that children with the highest enteric enteropathy (EE) score (calculated using the three fecal biomarkers neopterin, myeloperoxidase and alpha-1-antitrypsin) grew 1.08 cm less than those with the lowest EE score during the following 6-month period.[4] EED is of greater concern in infants born preterm or low birth weight, who are at higher risk of enteric infections and growth faltering.[5, 6, 7]

It is unclear whether EED, gut inflammation, or permeability can be prevented early in life. It is speculated that antibiotics or probiotics, improved infant feeding, zinc supplementation, or water, sanitation, and hygiene (WASH) interventions might improve gut function by reducing the enteric pathogen load, gut inflammation and/or permeability.[1] However, studies have not been able to demonstrate clear reproducible changes in fecal markers of gut function following these interventions.[8, 9, 10, 11] The World Health Organization (WHO) and the Government of India recommend Kangaroo Mother Care (KMC), an intervention encompassing skin-to-skin contact (SSC) and exclusive breastfeeding, to improve survival in low birth weight (LBW) babies.[12, 13, 14] A large randomized controlled trial in India among 8402 LBW infants demonstrated that promotion of community-initiated KMC (ciKMC) improved post-enrolment neonatal survival by 30%.[15] In addition, it reduced the risk of possible serious bacterial infection, diarrhea, and severe underweight during the neonatal period, i.e. the first 28 days of life. It is plausible that KMC reduces gut inflammation and permeability in LBW infants as the baby is placed in a

protective environment in SSC and exclusively breastfed, reducing the chance of enteropathogen exposure and clinical infection.

Our primary objective was to estimate the effect of ciKMC promotion in LBW infants on gut inflammation and permeability at the end of the neonatal period. In addition, we estimated the effect of ciKMC promotion on enteric enteropathy. We hypothesized that the promotion of ciKMC reduces gut inflammation and permeability as reflected in a lower concentration of fecal neopterin, myeloperoxidase, and alpha-1-antitrypsin, and enteric enteropathy as reflected in a lower composite EE score and EE index.

METHODOLOGY

Ethics Statement

Ethics approval was obtained from the Society for Applied Studies' ethics committee (SAS/ERC/KMCS/2017) and the Regional Committee for Medical and Health Research Ethics (REK) in Western Norway. The study was registered with Clinical trials registry-India (CTRI/2017/04/008430). Written informed consent including permission for storage of specimens for future research was obtained from the mothers of the eligible infants before enrollment.

Study design and participants

This individually randomized clinical trial was developed as a sub-study within a larger primary trial (ClinicalTrials.gov NCT02653534).[15, 16] The trial was conducted in rural and semi-urban low-income populations of Faridabad and Palwal districts in Haryana, India. As part of the primary trial[15, 17], pregnant women were followed up by a surveillance team periodically till delivery. Newborns weighing 1500 to 2250 g and their mothers were eligible for inclusion if they were

screened within 72 hours of birth. Infants were excluded if KMC had already been initiated in a birth facility, or infants were unable to feed, had breathing problems, gross congenital malformations, less than normal movements, or mothers were not living with their babies or intending to move away over the next six months. Additionally, in this sub-study, we excluded twins and triplets. In the primary trial enrolments were done between July 2015 to October 2018. For evaluation of fecal biomarkers, we enrolled consecutive 200 infants from May 2017 onwards who provided consent; only one eligible child was enrolled from each household (Figure 1).

Intervention and Usual Care

The ciKMC intervention comprised of promotion and support of continuous and prolonged SSC and exclusive breastfeeding. The intervention delivery team visited homes of the infant-mother dyads allocated to the ciKMC trial arm to initiate and support KMC. The team visited on days 1, 2, 3, 5, 7, 10, 14, 21, and 28 after birth to observe and solve any problems related to KMC Mothers and family members were taught to daily record the duration of SSC. Mothers were counselled to practice SSC for as long as possible during day and night, with the assistance of other family members. Visits continued till 28 days of age or if the baby wriggled out of KMC position and no longer accepted SSC, whichever was earlier. Referral of ill infants in both trial arms was facilitated through government Accredited Social Health Activists (ASHAs).[18] All infants in the intervention and control arms received usual care, i.e. home-based postnatal care visits by ASHAs as implemented through the health system.[19]

Study Outcomes

The primary outcomes were the concentration of the individual fecal biomarkers viz. neopterin (nmol/L), myeloperoxidase (ng/mL), and alpha-1-antitrypsin (µg/mL) at the end of the neonatal period (day-28 of birth up to plus 7 days). Other outcomes were EE score and EE index which is

a composite score calculated using three fecal biomarkers neopterin, myeloperoxidase and, alpha-1-antitrypsin. Neopterin is an indicator of T-helper cell 1 activity. Myeloperoxidase reflects neutrophil activity in the intestinal mucosa while alpha-1-antitrypsin indicates intestinal permeability and protein loss. These fecal biomarkers have been previously used in multiple studies as proxy measures of gut inflammation and permeability.[3, 20, 21, 22]

Assessment of Outcomes

Stool specimens were collected at baseline within the first week of birth and within 1 week after the end of the neonatal period. A stool kit consisting of a cold box with 4 ice-packs, a labelled sterile (gamma-irradiated) stool container with a spatula, a plastic nappy, and tissue paper was provided to the participant's mother. The process of specimen collection was demonstrated to the mother and family members by the field worker. The mother was instructed to store ~5g stool in the cold box only between 6 AM and 3 PM to enable prompt transportation to the laboratory. The mother called the study team upon specimen collection, after which a fieldworker transported the cold box to reach the Clinical and Research Laboratories, Society for Applied Studies (CRL SAS), New Delhi within 6 to 8 hours. The specimens were stored without fixatives[4] in a -80ºC freezer until analysis.

Laboratory analysis was initiated in CRL SAS, New Delhi, in October 2018. Fecal biomarkers of gut function were analyzed by the ELISA method using the automated EVOLIS™ Twin Plus system (BioRad, California, USA). We used IBL International Kit (Hamburg, Germany) for neopterin assessment; K6630 IDK MPO ELISA kit (Immundiagnostik AG, Bensheim, Germany) for myeloperoxidase; and the Human A1AT kit (Immuchrom Gmbh, Heppenheim, Germany) for alpha-1-antitrypsin. All three kits were verified to identify the acceptable range of values for accuracy using the manufacturer standards, inter and intra-assay precision, and linearity before conducting the experiments with the study stool specimens. Standard kit instructions were

followed; specimens with values out of range were diluted as required and the dilution factor was accounted for when calculating the final values.

Sample Size

With 95% confidence and 90% power, a total sample size of 168 infants (84 in each arm) was deemed sufficient to detect at least a 0.5 SD difference in the mean concentration of fecal biomarkers between the trial arms. Assuming a 15% attrition due to loss to follow-up or failed stool specimen collection or processing, we enrolled 200 infants (100 in each of the ciKMC and control arms) into our trial.

Statistical Analysis

Analyses were conducted on an intent-to-treat basis using STATA version 16 (Stata Corporation, College Station, TX). Given the right-skewed distribution of the fecal biomarkers, we reported medians with interquartile ranges (IQRs), presented violin plots for both study arms, and log-transformed (natural logarithm) the data prior to statistical analyses. Pearson correlation coefficient in pairwise comparisons was estimated for the three fecal biomarkers.

Using the approach of MAL-ED investigators[4], a composite score based on the percentile category of the myeloperoxidase, neopterin, alpha-1-antitrypsin concentrations was developed using the weightage factor as per principal component analysis. The principal component analysis indicated a 6-fold higher weight for myeloperoxidase and neopterin compared to alpha-1-antitrypsin. The EE score calculation is shown in the equation below, where myeloperoxidase, neopterin, alpha-1-antitrypsin categories are defined as 0 (≤ 25th percentile), 1, (25–75th percentile), or 2 (≥ 75th percentile). The score ranged from 0 to 26.

EE score = 6(neopterin category) + 6*(myeloperoxidase category) + 1*(alpha-1-antitrypsin category)*

To calculate the EE index we used Stata's "factor" command including the three log-transformed fecal biomarker variables and thereafter generated the index using the "predict" command. The value of this index ranged from -4.3 to 1.73.

We applied the Student's t-test to estimate if there were any substantial differences in the unadjusted mean of the log-transformed concentrations of the fecal biomarkers at 1 month of age between the ciKMC arm and the control arm. We used multivariable generalized linear models (GLM) of the Gaussian family with an identity link to estimate the difference in means of the log-transformed fecal biomarkers between the trial arms adjusted for its baseline concentration and unequally distributed potential confounding factors at baseline. Unequal distribution of a potential confounding factor was *a priori* defined as a relative difference of more than 10% across the study arms.[23] Wealth quintiles, WASH factors (toilet facility and source of drinking water), birth order, baby sex, weight at enrolment, and gestational age were the potential confounders associated with the primary outcome at $P < 0.10$ in univariable analysis.

Subgroup analyses, decided *a priori*, were conducted to estimate whether the effect of ciKMC on gut inflammatory biomarkers at the end of 1 month after birth was different in preterm infants (<37 weeks gestation) compared to term infants (≥37 weeks gestation). Gestational age was estimated from the ultrasonography reports, when available, or based on the last menstrual period as documented in hospital records or as per maternal recall, in the given order of preference.

RESULTS

Stool specimens were collected from all 200 infants enrolled in the trial at baseline and the end of the neonatal period. Fecal biomarker assessments were completed in 99% to 100% of the infants in both study arms for all three biomarkers (Figure 1).

The mean (SD) age when the baseline stool specimens were collected was 4.2 (1.6) days in the ciKMC arm and 5.1 (1.2) days in the control arm. The mean (SD) age of stool specimen collection at the end of the neonatal period was 31.6 (3.4) days in the ciKMC arm and 32.0 (5.5) days in the control arm. Baseline characteristics were similar in the two study arms other than for wealth quintiles (lower 3 quintiles), availability of toilet facility in the household, source of drinking water (public tap), birth order (≥5), and sex of the baby, where the relative differences between study arms exceeded 10% (Table 1).

All mothers in the intervention arm and 4% in the control arm reported practice of SSC during the neonatal period. In the intervention arm, the median (IQR) age of the infant at ciKMC initiation was 27.5 (12.5 to 38.5) hours. The mothers in the intervention arm practiced SSC for a median of 28 days with a mean (SD) of 12.2 (3.1) hours per day. Exclusive breastfeeding prevalence (24-hour recall) at day-28 was 84% in the ciKMC arm and 60% in the control arm (Online supplemental table 1). Diarrhea or dysentery during the neonatal period was reported in 3% in the ciKMC arm and 9% in the control arm infants. The chi-square test showed that there were no significant differences in diarrhea or dysentery between the trial arms (p=0.075)

Pearson's correlation coefficient (*r*) suggested a low correlation between the baseline concentrations of neopterin or myeloperoxidase with alpha-1-antitrypsin (r = <0.1). At baseline, the median (IQR) concentrations of fecal neopterin were 1497 (993 to 2397) nmol/L and 1268 (879 to 1893) nmol/L, myeloperoxidase were 201 (94 to 388) ng/mL and 208 (98 to 340) ng/mL,

and alpha-1-antitrypsin were 282 (148 to 544) µg/mL and 302 (187 to 549) µg/mL in the ciKMC arm and control arm infants, respectively (Figure 2).

At the end of the neonatal period, the median (IQR) concentration of fecal neopterin were 1866 (1022 to 2371) nmol/L and 1689 (1055 to 2355) nmol/L; myeloperoxidase were 324 (137 to 498) ng/mL and 262 (93 to 392) ng/mL; alpha-1-antitrypsin were 310 (164 to 649) µg/mL and 298 (178 to 605) µg/mL in the ciKMC and control arm infants, respectively (Figure 3). The mean (SD) composite EE Score was 13.6 (7.5) in the ciKMC arm and 12.4 (8.3) in the control arm infants.

The adjusted difference in means between the ciKMC arm and control arm in the log-transformed concentration of neopterin was 0.03 (95% CI -0.15 to 0.21), myeloperoxidase was 0.28 (95% CI -0.05 to 0.61), and alpha-1-antitrypsin was 0.02 (95% CI -0.30 to 0.34). The adjusted difference in means in the EE score was 0.44 (95% CI -1.81 to 2.69), that for the EE index 0.17 (95% CI -0.11 to 0.45). Unadjusted analysis showed similar results (Table 2).

In term infants, there was virtually no difference between the study arms in the mean log-transformed concentrations of fecal biomarkers, nor in the EE score or the EE index (Table 3). Among the preterm infants, the adjusted difference in means between study arms in the log-transformed concentration of myeloperoxidase was 0.41 (95% CI 0.02 to 0.82), and that for EE index was 0.38 (95%CI 0.01 to 0.75).

DISCUSSION

We aimed to estimate the effect of ciKMC promotion among LBW infants on fecal biomarkers of gut inflammation and permeability, enteric enteropathy score and index at the end of the neonatal period. In our trial of 200 North Indian LBW infants, we did not find evidence of any

246 substantial effect of ciKMC promotion on the concentrations of fecal neopterin,
247 myeloperoxidase, or alpha-1-antitrypsin, nor on the enteric enteropathy score or index.

248 Our effectively implemented randomization, no loss to follow-up and adjustment for potential
249 confounders makes it unlikely that selection bias compromised the validity of our findings. Errors
250 in the measurement of fecal biomarkers are unlikely, given the use of an automated ELISA
251 system and pre-study kit validation exercises. Similar to the protocol followed in the MAL-ED
252 study sites[4], we did not use any fixatives or protease inhibitors while storing fecal specimens.
253 Therefore, we cannot rule out the possibility of some degree of natural degradation of the
254 biomarker proteins. Nonetheless, given that the fecal specimens were stored within 6-8 hours of
255 stool passage at -80ºC, with no freeze-thaw events, we believe this not to be a major concern.
256 There is a possibility that stool specimens collected during a diarrheal episode may lead to
257 inaccurate measurement of fecal biomarker concentrations. We included all eligible LBW infants
258 in the primary analysis, as history-based ascertainment of diarrhea in neonates is not always
259 reliable. Although we did not find a statistically significant difference in this substudy, the
260 proportion of children with diarrhea or dysentery in the trial arms was comparable to that in the
261 primary trial.[15] A sensitivity analysis excluding the infants with diarrhea during the neonatal
262 period showed similar estimates as described in the results section (data not shown). With the
263 low likelihood of bias, the study findings seem internally valid and suggest that the promotion of
264 ciKMC is unlikely to affect gut inflammation and permeability in the target population of stable
265 LBW infants in our study setting.

266 We did not find previous studies that examined the effect of KMC on infant gut function. Some
267 trials evaluated the effect of different interventions like WASH or improved infant feeding
268 practices on gut function but failed to demonstrate clear effects on reducing gut inflammation or
269 permeability.[8, 22] The SHINE l cluster-randomized trial, estimated the effect of improved WASH
270 and infant and young child feeding (IYCF) practices on environmental enteric dysfunction in

children aged 1 to 18 months.[8] The trial found no effect of improved WASH or IYCF interventions on fecal myeloperoxidase, neopterin, alpha-1-antitrypsin levels in the first 6 months of life. Another trial in Bangladesh showed that age-appropriate nutrition counselling plus a lipid-based nutrient supplement substantially reduced neopterin concentration at 3 and 14 months of age, but there was no effect on myeloperoxidase, alpha-1-antitrypsin levels, or lactulose-mannitol ratio, another marker of gut permeability. At 28 months of age, however, myeloperoxidase and the lactulose-mannitol ratio were higher among children in the intervention arm than in the control arm.[22] Our trial did not show evidence of any effect of KMC promotion on fecal biomarker concentration at the end of the neonatal period. Better resource availability could have enabled a longer follow-up period, which would have been useful to study the effect over time.

The median concentrations of myeloperoxidase, neopterin, and alpha-1antitrypsin in our study were lower than that observed in many of the previous studies. This may be because of the specific population of LBW infants included in our trial, the timing of fecal specimen collection and/or differences in the ELISA kits used. We analysed the concentration of fecal specimens which were collected within 7 days of birth and at the end of the neonatal period, whereas in earlier studies fecal specimens were studied mostly in children 3 months and older.[4, 21, 24, 25] A study documenting trends in fecal biomarker concentrations over time suggests that they are probably higher when babies are 3 to 6 months of age than younger infants.[24]

The exploratory subgroup analyses in preterm infants did not suggest any meaningful differences between the study arms in the measured fecal biomarkers at the end of the neonatal period. The somewhat higher myeloperoxidase levels among preterm infants in the ciKMC arm could be an incidental finding.[26] Alternatively, it may be explained by the higher rates of exclusive breastfeeding in the ciKMC arm which are believed to be associated with increased

fecal myeloperoxidase levels.[24] More research on the effect of interventions on gut function in preterm infants and association of breastfeeding with fecal biomarkers of inflammation would be helpful.

The study did not show evidence to support our hypothesis that KMC promotion can reduce fecal biomarkers of gut inflammation and permeability in stable LBW neonates in rural and peri-urban settings in North India. Our study had some limitations. The findings may not be generalizable to different settings, or among unstable or very low birth weight infants. Additional information on maternal nutrition parameters that might influence infant gut function could be useful to document adequate randomization and contextualize our findings. Further research is needed to substantiate our findings and to study if the intervention has an impact on EED, which is a broader entity encompassing several aspects of gut function and systemic inflammation.[27] Biomarkers of EED are seen to be associated with the presence of multiple viral and bacterial pathogens (enteroviruses, adenoviruses, *Campylobacter* spp., and diarrheagenic *Escherichia coli*) in the gut.[25] Future assessment of intervention effects on gut function may consider detection and quantitation of fecal enteropathogens in addition to the biomarkers. The fact that promotion of ciKMC reduced the risk of severe neonatal stunting and wasting[15], yet in the current study we did not find a measurable effect on fecal biomarker concentrations, underscores the need to look for additional mechanisms that can explain growth faltering. Because EED may be influenced by multiple factors it may be worthwhile to explore the role of integrated health interventions on gut function in LBW infants.

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Drafting of the manuscript: Sinha, Sommerfelt, Ashorn

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Statistical analysis: Sinha, Sommerfelt, Ashorn

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Table 1. Baseline characteristics of the study participants*

Variables	Control arm (n=100) %	ciKMC arm (n=100) %
Household characteristics		
Wealth Quintiles		
Least poor	19	16
Less poor	25	27
Poor	17	26
Very poor	25	13
Most poor	14	18
Religion		
Hindu	86	85
Muslim	10	14
Others	3	1
Type of family		
Nuclear	33	35
Joint	67	65
Toilet not available inside household	17	13
Source of drinking water		
Piped water	20	19
Tube well	31	33
Public tap	28	22
Bottled water	19	25
Other	2	1
Mean number of family members (SD)	7.0 (2.8)	7.6 (3.7)
Maternal and paternal characteristics		
Mean maternal age: years (SD)	24.1 (3.7)	23.1 (3.2)
Mean paternal age: years (SD)	27.2 (4.8)	26.4 (4.2)
Maternal education: years of schooling (SD)	7.1 (5.3)	5.7 (5.2)
Birth related characteristics		
Place of delivery: Home	14	16
Birth order		
1	35	37
2-4	56	56
≥5	9	7
Infant characteristics		
Sex of the baby: Female	52	59
Mean weight at enrolment in gm (SD)	2094.5 (162.1)	2086.2 (139.1)
Mean gestational age in weeks (SD)**	35.9 (1.9)	35.9 (1.6)
Preterm <37 weeks	62	63

*Data presented are number/ percentages unless indicated otherwise

**67.5% (135/200) had an ultrasound for gestational age assessment

Table 2. Fecal biomarker concentration, enteric enteropathy (EE) score and EE index among study participants at the end of the neonatal period

Fecal biomarkers	**Control arm**	**ciKMC arm**	**Unadjusted** Difference in means (95% CI)	**Adjusted**[†] Difference in means (95% CI)
Log Neopterin nmol/L				
Mean (SD)	7.30 (0.68)	7.37 (0.61)	0.07 (-0.11 to 0.25)	0.03 (-0.15 to 0.21)
Log Myeloperoxidase ng/mL				
Mean (SD)	5.12 (1.29)	5.49 (1.01)	0.38 (0.05 to 0.70)	0.28 (-0.05 to 0.61)
Log Alpha1antitrypsin µg/mL				
Mean (SD)	5.57 (1.09)	5.61 (1.16)	0.04 (-0.27 to 0.35)	0.02 (-0.30 to 0.34)
EE score				
Mean (SD)	12.36 (8.32)	13.57 (7.51)	1.21 (-1.01 to 3.43)	0.44 (-1.81 to 2.69)
EE index				
Mean (SD)	-0.12 (1.12)	0.12 (0.86)	0.25 (-0.03 to 0.53)	0.17 (-0.11 to 0.45)

[†] Adjusted for potentially confounding baseline factors when the relative difference at baseline between trial arms were >10%, i.e., wealth quintiles, toilet facility, source of drinking water, birth order, sex of the baby, and baseline concentration of the respective gut inflammatory markers.

Table 3. Effect of ciKMC on infant fecal biomarkers at the end of the neonatal period in subgroups of preterm and term infants

Gut Biomarkers at 1 month	**Infant subgroup**	**Control arm** Mean (SD)	**ciKMC arm** Mean (SD)	**Unadjusted** Difference in means (95% CI)	**Adjusted** † Difference in means (95% CI)
Log Neopterin nmol/L	Preterm (n=125)	7.27 (0.75)	7.43 (0.61)	0.17 (-0.07 to 0.41)	0.16 (-0.09 to 0.40)
	Term (n=75)	7.37 (0.57)	7.27 (0.60)	-0.10 (-0.37 to 0.17)	-0.26 (-0.55 to 0.04)
Log Myeloperoxidase ng/mL	Preterm (n=125)	5.13 (1.13)	5.51 (0.98)	0.39 (0.01 to 0.76)	0.41 (0.02 to 0.82)
	Term (n=75)	5.11 (1.54)	5.47 (1.09)	0.36 (-0.25 to 0.98)	0.01 (-0.58 to 0.60)
Log Alpha1antitrypsin µg/mL	Preterm (n=125)	5.39 (1.24)	5.55 (1.23)	0.16 (-0.28 to 0.60)	0.24 (-0.21 to 0.69)
	Term (n=75)	5.86 (0.74)	5.70 (1.02)	-0.16 (-0.57 to 0.25)	-0.21 (-0.66 to 0.24)
EE Score at 1 month	Preterm (n=125)	11.7 (7.9)	14.2 (7.6)	2.5 (-0.27 to 5.26)	2.5 (-0.40 to 5.36)
	Term (n=75)	13.5 (8.9)	12.5 (7.2)	-0.99 (-4.77 to 2.80)	-3.84 (-7.76 to 0.08)
EE index at 1 month	Preterm (n=125)	-0.18 (1.1)	0.19 (0.8)	0.37 (0.02 to 0.72)	0.38 (0.01 to 0.75)
	Term (n=75)	-0.04 (1.1)	0.01 (0.8)	0.04 (-0.43 to 0.51)	-0.29 (-0.76 to 0.18)

† Adjusted for potentially confounding baseline factors i.e., wealth quintiles, toilet facility, source of drinking water, birth order, sex of the baby, and baseline concentration of the respective gut inflammatory markers.

Figure 1. Participant flow in the trial

Figure 2. Fecal biomarkers of inflammation and permeability across study arms at baseline

Figure 3. Fecal biomarkers of inflammation and permeability across study arms at the end of the neonatal period

PUBLICATION III

Effect of Community-Initiated Kangaroo Mother Care on Postpartum Depressive Symptoms and Stress Among Mothers of Low-Birth-Weight Infants: A Randomized Clinical Trial

Bireshwar Sinha, Halvor Sommerfelt, Per Ashorn, Sarmila Mazumder, Sunita Taneja, Deepak More, Rajiv Bahl, Nita Bhandari

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Original Investigation | Psychiatry

Effect of Community-Initiated Kangaroo Mother Care on Postpartum Depressive Symptoms and Stress Among Mothers of Low-Birth-Weight Infants
A Randomized Clinical Trial

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Abstract

IMPORTANCE Approximately 1 in 5 women in low- and middle-income countries experience postpartum depression, and the risk is higher among mothers of low-birth-weight (LBW) infants. Kangaroo mother care (KMC) is effective in improving survival among LBW infants, but the benefits of KMC for mothers are not well described.

OBJECTIVE To estimate the effects of community-initiated KMC (ciKMC) on maternal risk of moderate-to-severe postpartum depressive symptoms and on salivary cortisol concentration, a biomarker of stress.

DESIGN, SETTING, AND PARTICIPANTS This was an unmasked, parallel-group, individually randomized clinical trial. Participants included 1950 mothers of stable LBW infants (weighing 1500-2250 g) in rural and semiurban low-income populations in North India enrolled between April 2017 and March 2018. Data analysis was performed from January to July 2020.

INTERVENTIONS Eligible participants were randomly assigned to the intervention or control group by block randomization. The mothers in the intervention group were supported to practice ciKMC until 28 days after birth or until the infant wriggled out of the KMC position (ie, was no longer staying in the KMC position). The intervention included promotion and support of skin-to-skin contact and exclusive breastfeeding through home visits.

MAIN OUTCOMES AND MEASURES Postpartum depressive symptoms at the end of the neonatal period were measured using the Patient Health Questionnaire-9, with a score of 10 or higher used to identify moderate-to-severe depressive symptoms. Salivary cortisol concentration was measured in a subsample of 550 mothers before and after breastfeeding on day 28 after birth.

RESULTS Of the 1950 participants (mean [SD] age, 23 [3.5] years), outcome assessment was completed for 974 of 1047 participants (93%) in the intervention group and 852 of 903 participants (94%) in the control group. Sixty-four percent of participants (1175 of 1826 participants) belonged to the lowest 3 wealth quintiles. The proportion of mothers with moderate-to-severe postpartum depressive symptoms was 10.8% (95% CI, 8.9%-12.9%; 105 of 974 mothers) in the intervention group vs 13.6% (95% CI, 11.4%-16.1%; 116 of 852 mothers) in the control group. The adjusted relative risk of moderate-to-severe maternal postpartum depressive symptoms was 0.75 (95% CI, 0.59-0.96), or an efficacy of 25%. There was no difference in day-28 salivary cortisol concentration between the ciKMC and control group mothers before or after breastfeeding. The analysis estimated that supporting 36 mothers to perform KMC at home would prevent 1 mother from experiencing moderate-to-severe postpartum depressive symptoms.

(continued)

Key Points

Question Does the practice of community-initiated kangaroo mother care (ciKMC), an intervention encompassing skin-to-skin-contact and exclusive breastfeeding, during the neonatal period reduce the risk of moderate-to-severe postpartum depressive symptoms among mothers of low-birth-weight (LBW) infants?

Findings In a randomized clinical trial that included 1950 mothers of stable LBW infants from low-income areas in India, the practice of ciKMC resulted in a 25% reduction in the relative risk of moderate-to-severe depression at 4 weeks after delivery. The analysis estimated that supporting 36 mothers to perform KMC at home would prevent 1 mother from experiencing moderate-to-severe postpartum depressive symptoms.

Meaning These findings suggest that ciKMC can have substantial benefits for maternal mental health, beyond improving the survival of LBW infants.

+ Visual Abstract

+ Supplemental content

Author affiliations and article information are listed at the end of this article.

Abstract (continued)

CONCLUSIONS AND RELEVANCE These findings suggest that ciKMC practice may substantially reduce the risk of moderate-to-severe maternal postpartum depressive symptoms. This evidence supports KMC as an intervention to be incorporated in essential newborn care programs in low- and middle-income settings.

TRIAL REGISTRATION Clinical Trials Registry–India Identifier: CTRI/2017/04/008430

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Introduction

Postpartum depression[1,2] affects quality of life and long-term psychological health in mothers and can also adversely affect mother-child interaction, breastfeeding, infant growth, and development.[3-7] Pooled estimates from 53 studies in 23 low- and middle-income countries reported the prevalence of postpartum depression to be 19% (95% CI, 16%-23%).[8] A meta-analysis[9] that included 38 studies in India estimated the prevalence of postpartum depression to be 22% (95% CI, 19%-25%). The prevalence of depression is reported to be higher among mothers of preterm compared with full-term infants in the first 12 weeks after birth.[10,11]

Several psychosocial and psychological interventions have been evaluated for their effect on the risk of postpartum depression. These include postpartum home visits by professionals, telephone-based peer support, and interpersonal psychotherapy. Although promising, they are not widely implemented in the Indian public health system.[12] Kangaroo mother care (KMC), an intervention encompassing skin-to-skin contact (SSC) and exclusive breastfeeding, reduces the risk of death and severe infection in low-birth-weight (LBW) infants and is recommended by the World Health Organization and the Government of India.[13-15] In a large randomized clinical trial[16] in India among 8402 LBW infants, promotion of community-initiated KMC (ciKMC) improved postenrollment neonatal survival by 30% and infant 6-month survival by 25%. KMC could reduce the risk of postpartum depressive symptoms through mother-infant bonding and possibly via the release of maternal oxytocin and lowering of cortisol secretion.[17-19] Data from observational and quasi-experimental studies[20-23] suggest such a beneficial effect on mothers, but conclusive evidence is lacking.

Our primary objective was to test the hypothesis that the practice of ciKMC during the neonatal period can reduce the risk of moderate-to-severe maternal postpartum depressive symptoms. As a secondary objective, we estimated the effect of ciKMC on maternal salivary cortisol concentration, a biomarker of stress, at the end of neonatal period.

Methods

Study Design and Participants

This unmasked, parallel-group, individually randomized clinical trial was developed as a substudy within a larger primary trial where the effect of ciKMC on neonatal and early infant mortality was estimated (ClinicalTrials.gov identifier: NCT02653534).[16,24] Here, we report the outcomes related to maternal mental health (ie, postpartum depressive symptoms) and stress as measured by cortisol. The study was conducted in rural and semiurban low-income populations of Faridabad and Palwal districts in Haryana, India.

Ethics approval was obtained from the institutional ethics committee at the Centre for Health Research and Development, Society for Applied Studies, New Delhi, India, and the Regional Committee for Medical and Health Research Ethics in Norway. For eligible mothers, written informed consent was obtained in the local language prior to enrollment (see the Trial Protocol in

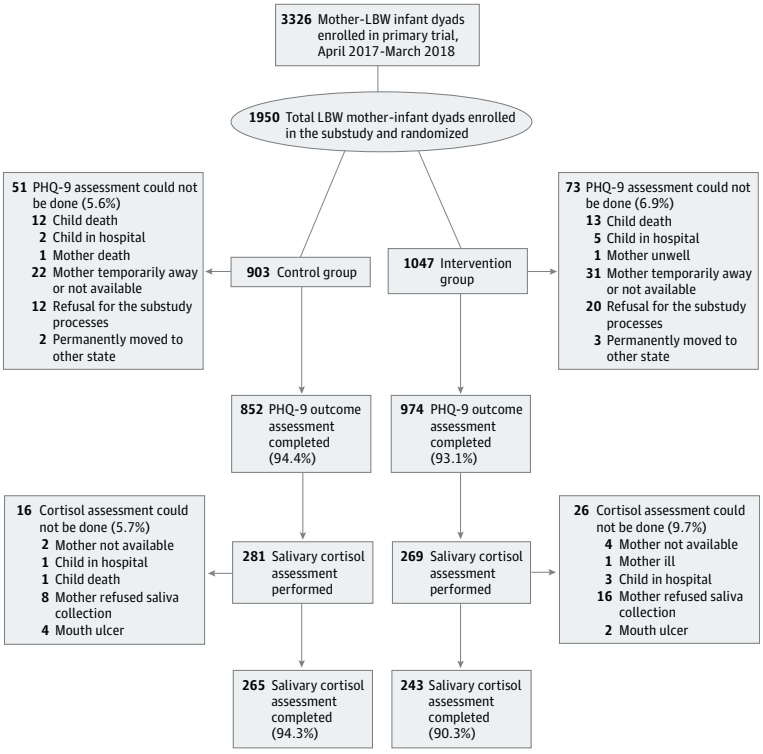
Supplement 1). The study is reported as per the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.[25]

As part of the primary trial, pregnant women were followed up by a surveillance team until delivery. Newborn infants weighing 1500 to 2250 g and their mothers screened within 72 hours of birth were eligible to be included in the trial, unless KMC had already been initiated in a birth facility or infants were unable to feed, had breathing problems, had gross congenital malformations, or had less than normal movements.[26] Mothers not living with their infants and those intending to move away over the next 6 months were excluded. In our substudy, we excluded mothers of twins or triplets. For feasibility reasons, we made an a priori decision to restrict enrollments to a maximum of 6 per day, randomly selected from those enrolled in the primary trial. Between April 2017 and March 2018, 1950 of 3326 mothers enrolled in the primary trial were included in this substudy for evaluation of postpartum depressive symptoms (**Figure 1**).

Randomization and Masking

The randomization list was prepared by an independent statistician using random permuted blocks of variable size. Allocation of participant identification number was done by an off-site randomization coordinator using serially numbered opaque sealed envelopes. To minimize contamination between trial groups, if a previously enrolled infant was allocated to the intervention group, the next eligible infant belonging to the same household was also allocated to intervention. If a previously enrolled infant was allocated to the control group, the next eligible infant in the same household was assigned

Figure 1. Flowchart of Participants



LBW indicates low birth weight; PHQ-9, Patient Health Questionnaire-9.

to intervention or control as per the randomization sequence. Further details of methods of the primary trial have been published elsewhere.[16,24]

Intervention and Usual Care

The ciKMC intervention comprised promotion and support of SSC and exclusive breastfeeding. Infant-mother dyads allocated to the ciKMC group were visited at home by an intervention delivery team consisting of a pair of trained workers to initiate and support KMC. The team observed breastfeeding and promoted SSC by using photographs and the local term *chaati se chipkaana*, which means *sticking the baby to the chest*. Mothers were counseled to practice SSC as long as possible during day and night with the assistance of other family members and were taught to daily record the duration of SSC. The team visited the families on days 1, 2, 3, 5, 7, 10, 14, 21, and 28 after birth to observe and address any problems related to SSC and/or breastfeeding; the duration of the visits ranged from 30 to 45 minutes. Visits continued until the infant wriggled out from the KMC position and no longer accepted SSC or until 28 days of age, whichever was earlier.[24,26] Referral of ill infants in both trial groups was facilitated through government-accredited social health activists. All infants in the intervention and control groups received usual care (ie, home-based postnatal care visits by accredited social health activists as implemented through the health system).[27]

Outcomes

Our primary outcome was moderate-to-severe postpartum depressive symptoms at the end of the neonatal period (day 28 after birth) measured using a pretested Hindi version of the validated Patient Health Questionnaire–9 (PHQ-9).[28,29] Secondary outcomes were any depressive symptoms, PHQ-9 score, and maternal salivary cortisol concentration at the end of neonatal period. Postpartum depressive symptoms were defined as PHQ-9 scores of 10 or higher for moderate-to-severe symptoms, 5 to 9 for mild symptoms, and 0 to 4 for no or minimal symptoms.[28] A PHQ-9 score of 5 or higher defined any depressive symptoms. The sensitivity and specificity of a PHQ-9 score 10 or higher to diagnose major depression in the postpartum period are reported to be approximately 80% and greater than 90%, respectively.[29,30]

Assessments

Study workers were trained in good clinical practice.[31] Reported duration of SSC and exclusive breastfeeding were captured by an independent outcome ascertainment team at the end of the neonatal period for all study participants. For the PHQ-9 interviews, an independent team of health workers was trained and assessed by a clinical psychologist. The PHQ-9 assessment was done after day 28, but not later than day 42 after birth. Supervisors conducted random quality checks in 1% of the assessments. Women with PHQ-9 scores 10 or higher were referred to health facilities.

We collected salivary specimens from the first 550 mothers on day 28 after birth to measure salivary cortisol. To account for diurnal fluctuations and variability related to breastfeeding, the specimens were collected before and after breastfeeding, not later than noon. The cortisol concentration was estimated by a Cortisol Enzyme Immunoassay Kit (Salimetrics) using an automated system (Twin Plus, Evolis) at Clinical and Research Laboratories, Society for Applied Studies, New Delhi. The kit performed within acceptable limits for accuracy, precision, and linearity for values between 1.0 µg/dL and 0.038 µg/dL (to convert cortisol to nanomoles per liter, multiply by 27.588). Specimens with values less than or equal to 0.037 µg/dL were assigned the lowest acceptable value.

Statistical Analysis

Assuming 90% power, 95% confidence, and 10% attrition and to be able to identify a minimum of 30% relative reduction from a 19% proportion of mothers with moderate-to-severe postpartum depressive symptoms in the control group,[9] the total sample size required was 1942 participants according to a 2-sided test (see the Statistical Analysis Plan in Supplement 1). Analyses were

conducted on an intent-to-treat basis using Stata statistical software version 16 (StataCorp). We estimated gestational age from ultrasonography reports, hospital records, or maternal recall, whichever were available, in the given order of preference.

We estimated relative risks (RRs) with 95% CIs for maternal moderate-to-severe or any depressive symptoms between the study groups using generalized linear models of the binomial family with a log-link. The efficacy of the intervention against maternal moderate-to-severe depressive symptoms was calculated as (1 − RR) × 100. We conducted multinomial logistic regression to estimate the effect of ciKMC on the risk of different categories of maternal depressive symptoms with no or minimal depressive symptoms (PHQ-9 scores of 0-4) as the reference. To compare salivary cortisol levels, we used the Wilcoxon rank-sum test.

Following CONSORT 2010 guidelines,[32] our approach was to present both unadjusted and adjusted results while avoiding overadjustment. Wealth quintile, mother age, mother education, and birth order were the potentially confounding covariates associated with the primary outcome at $P < .10$ in univariable analysis. To avoid overadjustment,[33] we included the potential confounding factors in the final multivariable regression model only if they were unequally distributed between the study groups at baseline, a priori defined as a relative difference of greater than 10%.[34] We used the likelihood ratio test based on deviance statistics to assess goodness-of-fit of the model[35] Design effects of infant-mother dyads enrolled from a single household were accounted for using Stata's robust variance estimator (vce) option. To quantify any biological interaction between ciKMC and preterm birth, we estimated the relative excess risk due to interaction[36] using the icp command.

We conducted exploratory subgroup analyses to estimate whether the effect of ciKMC on postpartum depressive symptoms was different in mothers with preterm birth (<37 weeks gestation) compared with full-term birth (≥37 weeks gestation). Data analysis was performed from January to July 2020.

Results

Of the 1950 enrolled participants (mean [SD] age, 23 [3.5] years), we completed PHQ-9 assessments for 93% of mothers (974 of 1047 participants) in the intervention group and 94% of mothers (852 of 903 participants) in the control group (Figure 1). In both study groups, 64% of participants (1175 of 1826 participants) belonged to the lower 3 wealth quintiles. Other baseline characteristics were well balanced across the study groups (**Table 1**) except for high birth order (ie, birth order ≥5), where the relative difference between study groups was 26%.

In the intervention group, 99% of the mothers (971 of 974 mothers) reported practicing SSC during the neonatal period vs 4% (33 of 852 mothers) in the control group. In the intervention group, the median (interquartile range) age of the infant at ciKMC initiation was 48 (23-72) hours; the mean (SD) duration of practicing SSC was 27.5 (3.9) days with a mean (SD) of 12.0 (3.7) hours per day. Approximately 2% of mothers continued SSC beyond the 28-day period. Exclusive breastfeeding prevalence (24-hour recall) at day 28 was 88% in the intervention group (859 of 974 participants) vs 57% (486 of 852 participants) in the control group. The number of home visits by accredited social health activists for postnatal care were similar in the 2 trial groups (eTable 1 in Supplement 2).

The proportion of mothers with moderate-to-severe postpartum depressive symptoms was 10.8% (95% CI, 8.9%-12.9%; 105 of 974 mothers) in the intervention group vs 13.6% (95% CI, 11.4%-16.1%; 116 of 852 mothers) in the control group (**Table 2**). The RR for moderate-to-severe postpartum depressive symptoms adjusted for birth order categories and taking household clustering into account was 0.75 (95% CI, 0.59-0.96), corresponding to an efficacy of 25% (95% CI, 4%-41%); the unadjusted RR was 0.79 (95% CI, 0.62-1.01). Additional adjustments for wealth quintile, mother age, and mother education changed the adjusted effect estimate only negligibly (data not shown). Multinomial regression showed a similar adjusted effect size of the intervention on the risk of moderate-to-severe depressive symptoms (RR, 0.73; 95% CI, 0.54-0.98), but no effect on mild depressive symptoms (RR, 1.00; 95% CI, 0.79-1.26) (eTable 2 in Supplement 2). The absolute risk

difference for moderate-to-severe postpartum depressive symptoms was 2.8% (95% CI, 0.1%-5.8%), corresponding to a number needed to treat of 36 mother-infant dyads (95% CI, 17-1000 dyads). The median (interquartile range) PHQ-9 scores were 2 (0-5) in the intervention group and 2 (0-6) in the control group. The cumulative frequency plot showed a left-shift of the PHQ-9 scores among ciKMC group compared with the control group mothers (**Figure 2**), suggesting that at any

Table 1. Baseline Characteristics of Participants in the Control and Intervention Groups

Variables	Participants, No. (%)	
	Control (n = 852)	ciKMC (n = 974)
Household characteristics		
Wealth quintiles, poor		
Least	146 (17.1)	167 (17.2)
Less	157 (18.4)	181 (18.6)
Poor	178 (20.9)	204 (20.9)
Very	204 (23.9)	229 (23.5)
Most	167 (19.6)	193 (19.8)
Family social class		
General	191 (22.4)	200 (20.5)
Other class[a]	389 (45.7)	434 (44.6)
Scheduled caste or tribe	272 (31.9)	340 (34.9)
Type of family		
Nuclear	250 (29.3)	312 (32.0)
Joint	602 (70.7)	661 (68.0)
Family members, mean (SD), No.	7.4 (3.2)	7.7 (3.5)
Maternal and paternal characteristics[b]		
Maternal age, mean (SD), y	23.1 (3.5)	23.4 (3.6)
Maternal education		
None	283 (33.2)	341 (35.0)
Duration of education, mean (SD), y	6.3 (5.2)	6.1 (5.2)
Mother working outside home	11 (1.3)	14 (1.4)
Father's age, mean (SD), y	26.5 (4.6)	26.7 (5.2)
Father's education		
None	128 (15.0)	150 (15.4)
Duration of education, mean (SD), y	8.5 (4.8)	8.3 (4.8)
Father not working	51 (6.0)	50 (5.1)
Birth-related characteristics		
Place of delivery		
Home	129 (15.1)	150 (15.4)
Government facility	511 (60.0)	569 (58.4)
Private facility	212 (24.9)	255 (26.2)
Cesarean delivery	18 (2.1)	18 (1.9)
Birth order[b]		
1	343 (40.3)	367 (37.7)
2-4	450 (52.8)	515 (52.9)
≥5	59 (6.9)	91 (9.4)
Infant characteristics		
Female	470 (55.2)	557 (57.2)
Weight at enrollment, median (IQR), kg	2.1 (2.0-2.2)	2.1 (2.00-2.2)
Weight at enrollment, kg		
1.50-1.79	44 (5.2)	52 (5.3)
1.80-1.99	160 (18.8)	182 (18.7)
2.00-2.25	648 (76.1)	740 (76.0)
Gestational age, mean (SD), wk[c]	35.8 (2.0)	35.8 (2.0)
Preterm <37 wk[c]	535 (62.8)	619 (62.8)

Abbreviations: ciKMC, community-initiated kangaroo mother care; IQR, interquartile range.

[a] Refers to any class not included in the general group or a scheduled caste or tribe.

[b] Baseline information on parental characteristics and birth order is not available for 1 participant in the intervention group.

[c] A total of 1253 of 1826 mothers (68.6%) underwent an ultrasonography.

given PHQ-9 score cutoff, a lower proportion of mothers in the ciKMC group were above the cutoffs (eTable 3 in Supplement 2).

In the subgroup of mothers with preterm births, the RR of moderate-to-severe postpartum depressive symptoms in the ciKMC group was 0.71 (95% CI, 0.52 to 0.96) vs 0.86 (95% CI, 0.56 to 1.34) among mothers with full-term infants. Preterm birth had a relative excess risk due to interaction with ciKMC for the study outcome of 0.19 (95% CI, −0.20 to 0.58).

Salivary cortisol measurements were done in 92.4% of the mothers (508 of 550 mothers) (Figure 1). The median (interquartile range) day-28 maternal salivary cortisol concentrations in both the ciKMC group and control group were similar before and after breastfeeding (**Table 3**).

Table 2. Effect of ciKMC on Maternal Postpartum Depressive Symptoms Among Study Participants

Outcome variable	Participants, No./Total No. (%)		RR (95% CI)	
	Control	ciKMC	Unadjusted	Adjusted[a]
Moderate-to-severe depressive symptoms[b]				
All mothers	116/852 (13.6)	105/974 (10.8)	0.79 (0.62-1.01)	0.75 (0.59-0.96)[c]
Subgroup				
Mothers of preterm infants	80/535 (15.0)	68/612 (11.1)	0.73 (0.55-1.00)	0.71 (0.52-0.96)
Mothers of full-term infants	36/317 (11.4)	37/362 (10.2)	0.90 (0.58-1.39)	0.86 (0.56-1.34)
Any depressive symptoms[d]				
All mothers	275/852 (32.3)	294/974 (30.2)	0.94 (0.82-1.07)	0.92 (0.81-1.05)
Subgroup				
Mothers of preterm infants	183/535 (34.2)	195/612 (31.2)	0.74 (0.55-1.00)	0.92 (0.78-1.09)
Mothers of full-term infants	92/317 (29.0)	99/362 (27.3)	0.90 (0.58-1.38)	0.91 (0.72-1.15)

Abbreviations: ciKMC, community-initiated kangaroo mother care; RR, relative risk.

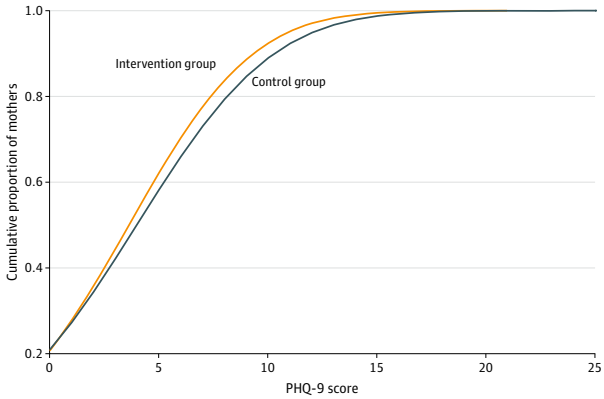
[a] Adjusted for birth order categories and accounting for household clustering.

[b] The primary outcome is moderate-to-severe postpartum depressive symptoms, which are defined as a Patient Health Questionnaire–9 score of 10 or higher.

[c] The adjusted model had an Akaike information criteria value of 0.73 and a bayesian information criteria value of −12352.16. The deviance statistic (G^2) is calculated to be 1322.33, with 1821 *df*. The G^2 corresponds to [−2 × (maximized log likelihood of the model of interest)] = [−2 × (−661.17)] and the *P* value referred to the deviance test is >.99, suggesting that the model is valid.[36]

[d] Any depressive symptoms are defined as a score of 5 or higher on the Patient Health Questionnaire–9. A total of 159 of 852 mothers (18.7%) in the control group and 189 of 974 mothers (19.4%) in the ciKMC group had mild depressive symptoms.

Figure 2. Cumulative Frequency Plot Showing Patient Health Questionnaire–9 (PHQ-9) Scores of Mothers in Control and Intervention Groups



Discussion

We found a 25% lower risk of moderate-to-severe postpartum depressive symptoms among mothers in the ciKMC group than among those in the control group. The RR reduction was somewhat higher among mothers of preterm infants. Our analysis indicated that ciKMC practice for approximately 36 stable LBW infant-mother dyads (ie, the number needed to treat) would prevent 1 case of moderate-to-severe postpartum depressive symptoms. We found no difference in the day-28 maternal salivary cortisol concentrations between the study groups.

Our study was done as a substudy within the primary ciKMC efficacy trial.[16] Selection and follow-up biases are not likely because of effective randomization, allocation concealment, and very low and balanced attrition. Nonetheless, we cannot rule out the possibility of recall bias. However, in the intervention group, information on the duration of SSC captured by the outcome assessment team based on mothers' history was similar to that obtained by the intervention delivery team during their scheduled visits, suggesting a low likelihood of such bias.[16] To minimize the possibility of conscious underreporting of symptoms, the outcome assessment team was extensively trained to conduct PHQ-9 assessment based on a comprehensive interview process in a comfortable home environment and not by asking the questions directly. Despite having an independent outcome assessment team, it might have been possible to guess the group allocation for those who practiced KMC beyond the 28-day period during PHQ-9 interview. However, such a possibility was small because only approximately 2% of mothers continued SSC beyond the 28-day period. Given the low risk of bias, the results are likely to be reliable and representative of the target population, suggesting that ciKMC can substantially reduce the risk of moderate-to-severe maternal postpartum depressive symptoms.

To the best of our knowledge, this is the first randomized clinical trial to estimate the effect of KMC on maternal depressive symptoms, and the findings substantiate those of previous reports from less rigorous study designs. In a quasi-experimental study in Canada,[20] depressive symptoms measured by the Edinburgh Postpartum Depressive Scale scores at 1 week and 1 month postpartum were lower among 30 mothers who practiced SSC compared with 60 mothers who did not. Another quasi-experimental study[23] of 50 Iranian mothers demonstrated that practice of KMC for 180 minutes daily for a week compared with when infants were cared for in incubators was associated with improved mean mental health scores assessed by the General Health Questionnaire. An observational study[21] in Portugal among 177 low-income mothers with preterm infants showed that the proportion of women with postpartum depression assessed by the Postpartum Depression Screening Scale decreased from 37% after delivery to 17% at hospital discharge with KMC practice. Our trial provides valid evidence of the efficacy of KMC on the risk of postpartum depression in mothers with LBW infants and substantiates the observations from the earlier studies.

KMC may lessen depressive feelings by empowering women in their mothering role and improving infant-mother bonding.[19,22,37] The substantial effect of ciKMC that we observed on postpartum depressive symptoms is likely to be a result of a multitude of complex psychological mechanisms, potentially supported and intensified by enhanced oxytocin release as a result of SSC

Table 3. Effect of ciKMC on Maternal Salivary Cortisol Levels Among Mothers at the End of The Neonatal Period[a]

	Salivary cortisol levels, median (IQR), μg/dL	
Time point	Control (n = 265)	ciKMC (n = 243)
Before breastfeeding	0.22 (0.16-0.31)	0.22 (0.17-0.29)[b]
After breastfeeding	0.18 (0.14-0.26)	0.19 (0.13-0.25)[b]

Abbreviations: ciKMC, community-initiated kangaroo mother care; IQR, interquartile range.

SI conversion factor: To convert cortisol to nanomoles per liter, multiply by 27.588.

[a] For estimation of salivary cortisol levels, 550 mothers were enrolled of whom we were able to collect salivary specimens from 508 (92.4%) mothers at day 28, before and after breastfeeding.

[b] Wilcoxon rank-sum test did not suggest any difference.

and exclusive breastfeeding.[17,38] Because of its clinical implications, we chose moderate-to-severe postpartum depressive symptoms as the primary outcome instead of PHQ-9 score. Women with PHQ-9 scores of 10 or higher have a high probability of major clinical depression that needs clinical attention, whereas the milder symptoms are often self-limiting.[28] In this context, our findings that ciKMC may reduce the risk of moderate-to-severe postpartum depressive symptoms are pertinent. The potentially greater benefit of ciKMC in mothers with preterm infants in reducing moderate-to-severe postpartum depressive symptoms is relevant given their higher risk of postpartum depression.[11] However, we acknowledge the limitations of our subgroup analysis, in that we did not stratify our randomization on whether the birth was preterm and because the statistical precision of the interaction was moderate.

Cortisol is a hormone produced by the activation of hypothalamic-pituitary-adrenocortical axis in response to physiological stress.[20,39] Salivary cortisol is widely used to capture short-term fluctuations in physiological stress.[40] The observed null effect of KMC on day-28 maternal salivary cortisol is similar to that of a quasi-experimental study in Canada,[20] where the mean cortisol concentrations among intervention and control mothers at the end of neonatal period were 0.23 and 0.24 μg/dL, respectively. These findings suggest a possible dissociation between acute stress and postpartum depression, as far as the effect of ciKMC is concerned. Further research on the effect of KMC on acute and chronic stress (using markers such as hair cortisol[40,41]) may be warranted.

Limitations

Although this is a large randomized clinical trial where almost all baseline characteristics were well-balanced between the trial groups, a baseline PHQ-9 assessment would have been valuable. Our study population was limited to mothers with stable LBW infants weighing 1500 to 2500 g, and the findings may not be applicable to mothers with unstable or very-LBW (ie, <1500 g) infants. Neonatal survival programs would benefit from further research that also includes such infants.

Conclusions

The findings of our study support ciKMC as an intervention to prevent maternal depressive symptoms in the early postpartum period. Research on long-term benefits of the intervention on maternal psychosocial health and child development would be helpful. The study findings together with previous literature[13,16,20,23] suggest substantial benefits of home-based KMC for mothers, beyond improving health and survival of LBW infants. Developing a focused LBW program is identified as one of the important agendas of the Indian Government[42] and maybe relevant in other low- and middle-income countries where a high proportion of infants are born with LBW. This evidence supports the integration of KMC into essential newborn care programs for LBW infant-mother dyads.

ARTICLE INFORMATION

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SUPPLEMENT 1.
Trial Protocol and Statistical Analysis Plan

SUPPLEMENT 2.
eTable 1. Practice of Kangaroo Mother Care Intervention Components and Home Visits Among Study Participants
eTable 2. Effect of ciKMC on Different Categories of Postpartum Depressive Symptoms Among Mothers at the End of the Neonatal Period
eTable 3. Proportion of Mothers With Postpartum Depressive Symptoms in Control and ciKMC Arm Using Different PHQ-9 Cutoff Scores

SUPPLEMENT 3.
Data Sharing Statement