Nutritional support for lactating women with or without Azithromycin for infants compared to breast-feeding counselling alone, to improve six-month growth outcomes among infants of peri-urban slums of Karachi, Pakistan – a protocol of multi-arm assessor blinded randomised controlled trial (Mumta LW trial)

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Study protocol

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Abstract

Background Globally, 45% of under-five deaths are, directly or indirectly, attributable to malnutrition, most of these deaths are in low- and middle-income countries (LMICs). Children in the first 6 months of life are particularly vulnerable. An estimated 4.7 million infants under the age of 6 months are moderately wasted whereas 3.8 million are severely wasted. Despite an increased risk to a child of a malnourished woman, there are discrepancies in guidance in this area.

Methods This is a community-based, open-label multi-arm factorial randomized controlled trial, using parallel assignment with 1:1:1 allocation ratio, in low-income squatter settlements of urban Karachi, Pakistan. In the control group (Control Arm A), women are randomized to standard counseling only; whereas in the first intervention group (Arm BIntervention arm 1), lactating women receive two sachets of balanced energy-protein (BEP) supplementation per day from enrollment till the infant reaches six months of age, in the second intervention group (Arm CIntervention arm 2), lactating women receive same BEP as in intervention Arm B while their babies also receive a single stat dose (20mg/kg orally) of azithromycin at 42 days. The primary outcome is relative length velocity from 0 to 6 months by the limb of allocation. The primary analysis will be Intention-to-treat analysis.

Trial registration Registration of the trial is done at ClinicalTrials.gov. NCT03564652, registered on June 21, 2018. Trial registration data is available through https://clinicaltrials.gov/ct2/show/NCT03564652

Background

Globally, about 45% of under-five deaths are attributable to childhood malnutrition, the main burden lying in low- and middle-income countries (LMIC). This is translated into an estimated three million under-five deaths annually rooted in undernutrition. (1) Children in the first 6 months of life are particularly vulnerable and an estimated 4.7 million infants under the age of 6 months are moderately and 3.8 million are severely wasted globally. (2) Breastfeeding confers protection, but in the face of maternal undernutrition, infection burden and competition with older siblings for breastfeeding due to short interpregnancy intervals, may not fully prevent undernutrition. (3) Lactating women in LMICs have a high prevalence of underweight themselves (4-7) and requires an additional 500 kcal per day as well as iron, iodine, and other micronutrients to maintain milk quality (8-10). Inadequate maternal dietary intake during pregnancy and lactation is the single strongest predictor of stunting and underweight among children. (11) The implications in terms of child growth, development and long-term health cannot be overstated. (12-14)

Despite this, there are limited randomized controlled trial evidences to guide intervention. In the intervention arm of a study in Ghana using small quantity Lipid-based Nutrient Supplements (SQ-LNS) iron and folic acid (IFA group), multiple micronutrients (MMN group) daily during healthy pregnancy until delivery and for 6 months postpartum, infants in LNS arm had statistically significantly greater length, length-for-age z-score (LAZ), weight, and weight-for-age z-score (WAZ) than other groups at 18 months of

age. (15) These findings, however, were not replicated by Lanou et al at Burkina Faso who used similar pre and postpartum interventions. (16) These studies were, however, not powered for analyzing the effect in undernourished women i.e. Mid-upper-arm-circumference (MUAC) of less than 23.0 cm or low Body Mass Index (BMI). Additionally, most of these trials where perinatal supplementation was given to the mother, showed that the impact of these supplements was not sustained during the infant period. (17) Though the MMN study showed only minimal differences in linear growth (18), there are theoretical reasons for enhancement with macronutrient supplements.

Further evidence is therefore required to assess the effectiveness of a balanced energy-high protein (BEP) supplement in undernourished lactating women in LMIC. Besides growth, fetal and infant nutritional compromise directly increases infection risk. (19) Interest in this area has been rekindled by the results of recent study involving mass Azithromycin prophylaxis to young children and a reduction in all-cause mortality. (20) Despite theoretical concerns regarding the development of bacterial resistance, there is no evidence so far of the emergence of such strains (20, 21). To understand the pathways of how these interventions might work is still not very clear i.e. if there is any impact on growth, what is the possible explanation? (21) To move further, we also want to access the impact of these, if any, on inflammatory biomarkers in both stool and blood, iron deficiency, and its impact on breastmilk's composition. The study will additionally enable us to examine changes in infant's stool microbiota which plays a substantial role in inflammation, immune development, enteropathy and in the nutritional –antimicrobial causal pathway. (22, 23, 24, 25). Therefore, a gap exists in the current knowledge that in the context where standard-of-care is 'only nutrition counseling to the undernourished lactating women', concerning whether a high dose BEP supplement (16-21 grams of protein per day) during lactation is beneficial to improve the infant's growth or not is yet to be determined. Additionally, a combination of BEP with a single prophylactic dose of Azithromycin to the infant of the same mother has any additional benefits is also yet to be studied. (20, 21) Further, in the context where stunting among the children under-five is more than 50%, our primary outcome of interest is length velocity over the period of the first 6 months.

Objectives

The primary objective is to compare the effect of a fortified, balanced energy-protein supplement (BEP) to lactating women's diet for 6 months (Intervention arm 1) with or without a single prophylactic dose of oral Azithromycin to the infant at 42 days of age (Intervention arm 2) with standard exclusive breastfeeding and nutritional counseling alone (Control arm) for improving length velocity among infants at 6 months (outcome). The secondary outcome is to compare the impact of interventions on weight (or growth) velocity, Z-scores, breast milk quality, gut microbiota, and key micronutrient level of both mother and infant.

Methods

Trial design

This is a multi-arm community-based randomized controlled, open-label but assessor blinded superiority trial with treatment allocation ratio of 1:1:1. Multi-arm trial is considered to understand the incremental impact of BEP to the mother, along with oral Azithromycin to the infants, on length velocity.

Study setting and study population

The trial is being conducted in the peri-urban communities of Karachi, Pakistan. These are impoverished coastal slums with a population of approximately 250,000 residents based on the census conducted in 2017. The annual birth cohort is around 5,000 each year. The population is multi-ethnic such as Sindhi, Pashtun, Punjabi, Bengali, and Urdu-speaking. Through a demographic surveillance system (DSS), bi-monthly visits are made to married women of reproductive age (13- 49 years) in the catchment area. During these visits, mortality, pregnancy, in and outmigration and the current number of under 5 children are recorded. From previous studies, there is a high prevalence of low birth weight (30%), stunting (52%) and wasting (18%) in the study areas. The study population is all lactating women of reproductive age who have recently delivered and their infants.

Participant eligibility criteria

The lactating women between 13 to 49 years of age and their newborn are enrolled, if they fulfill the inclusion and exclusion criteria provided in **Table 1**. Routine measurement of mid-upper arm circumference (MUAC) is done during surveillance rounds and women with MUAC of less than 23 cm in the first week of delivery are screened for eligibility by a research team. If eligible, the written informed consent is administered in the local language.

Table 1 Eligibility Criteria

Inclusion	Exclusion
Mid-Upper-Arm-Circumference of lactating woman < 23.0 cm	Birth weight of newborn is less than 1500 gram
Live birth outcome, captured within 168 hours	Newborn with known congenital anomaly or other serious illness based on study physician's assessment before enrollment.
Intention to stay in the catchment area for entire duration of trial after enrollment	Lactating women has known allergies to peanut, lentils, chickpea or dairy products
Intention to exclusively breastfeed child for at least 6 months of age	Previously enrolled in the trial
Voluntary written informed consent	

Sample size

There is limited data available on the impact of BEP on length velocity over the period of the first 6 months for an infant. However, in one of the studies, the mother who received only perinatal supplement showed that the overall increase in length (cm/month) among the LNS group was b=3.289, compared to b= 3.346 among the MMN group. (18) But, women in this trial received less dose/energy supplement, compared to what we are proposing, also, their intervention time is different i.e. perinatal compared to infancy. Another study showed that difference in length velocity (cm/month) was 0.02 among the MMN group compared to IFA over the period of 0-18 months. (19) Therefore, in the absence of clear evidence on the impact of these interventions, we hypothesized the effect size of 0.12cm/month to see the impact. This is also based on learning through field experience, where when provided severely malnourished lactating women (MUAC < 19.0 cm) with chickpea-based ready-to-use supplements (100 gm/day for 3 months), they showed a difference of 0.06 cm/month at 3 months of age compared to women who did not receive any supplement(data unpublished and purely from implementation, not a study).

The null hypotheses for this trial is that an infant of a lactating woman receiving standard breastfeeding counseling with BEP alone (Intervention arm 1) for 6 months or in combination with a single prophylactic dose of Azithromycin at 42 days of age (Intervention arm 2) has a mean difference in length velocity of less than 0.12 cm/month (primary outcome) compared to standard breastfeeding counseling alone (Control Arm). Learning through local experience from our field sites and due to absence of evidence of such intervention on length velocity, we used our field data. The alternate hypothesis for this trial is that an infant of a lactating woman receiving BEP alone for 6 months or in combination with a single prophylactic dose of Azithromycin at 42 days of age has a mean length velocity of greater than 0.12 cm/month or more compared to no intervention. The sample size takes multiple comparisons into account and is based on the primary outcome of length velocity with an effect size of 0.12 cm per month at least between the arm with a 1-sided test, 0.025 alpha to account for multiple comparisons (the lower alpha). A drop out of 14% (i.e. 10 % loss to follow up and 4% infants' deaths) is assumed in the study so a minimum total sample size required is 957 (319 LWs in each arm).

Recruitment

Leverage on existing married women surveillance system established by Department of Pediatrics and Child Health, Aga University, VITAL has access to line listing of all pregnant women at catchment area. Using these listings, the research team visits these households and built a rapport with pregnant women and leave our contact number to be used to notify birth events. During each pregnancy touchpoint, research team provides standard antenatal and nutrition counseling to each pregnant woman and guides

them for seeking proper care. At the time of birth, the randomization/enrollment team receives a birth notification so that team can visit the household for eligibility assessment.

Informed consent procedure

If eligible, a written informed consent is administered by the same team in a local language (mostly Urdu and where required in Sindhi and Pashto language). Team members explain details of the trial with purpose, follow-up procedures, specimen collections, and other related processes. If participant is eligible and agree for consent procedure to explain, the research team gives the consent to the participant or decision maker to read (if they can read) or team reads it word-by-word for them in Urdu or local language (if participant/decision maker cannot read). Participant are allowed to ask any question related to the consent and trial procedure. If participant/decision maker require further time to take more informed decision, team also allow this opportunity and wait until final voluntary decision is taken. After a voluntary agreement, consent is provided by the participant in the presence of a witness, either duly signed or thumb impression is provided by participant and the witness. Ethics committee has given the approval for thumb impression of the participant and witness if they cannot read or write. Only designated study team is involved in obtaining the written informed consent. A copy of informed consent is provided to all participants attached in the file with study ID. There is planned additional analysis, which may involve sending samples and data abroad. The consent document covers all the aspects of these procedures and participants have the opportunity to opt out from bio-banking or from participation for future research at any point of the trial. Still, any future secondary analysis will require institutional and national ethics committee approval.

Randomization and allocation concealment

After written informed consent procedure, randomization is performed by the team. Stratified block randomization with varying sized blocks of 3, 6, and 9 is used. Sequence generation is done by an independent statistician using a random selection method before the beginning of the trial. Self-adhesive, pre-coded sticking labels with the unique identification numbers are applied to sealed opaque envelopes containing coded information of randomization identification number and intervention to ensure that the randomization process and allocation is blinded. Baseline information is completed with counseling regarding nutrition and exclusive breastfeeding. Anthropometry of both mother and newborn is done and follow-up procedures is explained. Figure 1 shows the trial flow in detail.

Blinding

Outcome assessors are blinded, restricted only to anthropometry with a designated non-overlapping schedule with follow-up teams. All investigators are also blinded to group allocation throughout the period of the study. Further, independent statistician will perform the interim analysis for Data Monitoring and Safety Board (DSMB) blinded by arm. Further, data analyst who will perform the final analysis will be blinded and code will eventually unveil after sharing the blinded results with DSMB and investigators in a final review meeting.

Interventions

In the control arm, lactating women receives standard nutritional counseling and promotional messages of exclusive breastfeeding, a standard-of-care by a trained research team. In the ‘intervention arm 1’, in addition to above, lactating women receive 2 sachets of BEP supplementation per day until the infant reaches 6 months of age distributed by trained research team at enrollment and later on each follow-up visit. BEP is a certified product of the World Food Program and is locally produced by Ismail Industries, Karachi. The manufacturers do not and will not have a role in any part of the study. Each sachet contains a caloric value of 400 kilocalories per 75 grams and protein of around 10.5 grams. The source of protein is mainly chickpea, peanuts, lentils, legumes, and skimmed milk. In the ‘intervention arm 2’ lactating women receive standard nutritional counseling and promotional messages of exclusive breastfeeding along with BEP supplement (same as intervention arm 1) but in addition, their infants also receive a single prophylactic dose of Azithromycin oral suspension, 20 mg/kg at day 42 of life (window period of plus 7 days). All arms receive routine care including newborn care, immunization, counseling regarding newborn and infant care at home and timely referral to health facility in case of urgent need. All other non-study treatment such as medications, formula milk in infants etc. would be recorded on each follow-up. In case of any serious illness or any serious adverse event (reported or observed), intervention may hold or stop for limited period, after consultation with investigators and Data Safety and Monitoring Board.

Data collection and data management

Case report forms (CRFs) are designed to capture details on screening, eligibility, randomization, household demography, newborn assessment, danger signs, serious adverse events, compliance with intervention, exclusive breastfeeding, 24-hours food recall (to estimate usual intake as well as diversity on a monthly basis) and anthropometry. The data is collected on tablets with inbuilt logical check and skip patterns by trained team and updated on secure servers in real-time using digital application, which is design and built in-house. Auto alerts reminds about the schedule of follow up and data tracking is done on key indicators. All the data is collected in a real-time manner and uploaded on cloud server which is password protected and only accessible to the trial data management team and manager. Participants confidentiality is highly maintained through unique ID system and participants identification is not exposed to anyone outside the trial team. Tablets which are used, are password protected and only accessible to study team. Deidentified data will be used for analysis purpose.

Follow ups

Follow up teams perform home visits to provide counselling in all arms. BEP is also provided (intervention arm 1) and compliance measured through logging of number of empty sachets since last visit. Azithromycin 20mg/kg stat oral dose (intervention arm 2) is given to infants at day 42 of life. Assessment of compliance of exclusive breast feeding is done in all arms since the last visit. Follow-up is done daily for first 15 days, then at alternate days for 2 weeks, next every 72 hours for two weeks and finally weekly until child reaches the age of 6 months. Monthly 24-hour food recall data is also collected.

On each visit counseling is provided to the participants to reinforce adherence to BEP and exclusive breast feeding. For participant that shifts out of the catchment area, team has developed a system to follow them at new location, when possible. There is no plan of retention of these participants once their 6 months follow-ups are completed. However, through our existing free-of-cost primary health care facilities, the standard of care is available to all participants, even after completion of trial. After enrollment (within 168 hours of birth) total follow-up duration of each participant in the trial is 6 months. Figure 2 Time schedule of enrollment, interventions, and assessments.

Anthropometry

Teams are trained for anthropometry of infant and mother by a master trainer using INTERGROWTH-21st standards with monthly refreshers. The measurements include infant length, weight, MUAC and head circumference and maternal MUAC and weight. Two readers measure the infant and mother blinded from one another, data is entered in digital form and system calculates the average reading automatically. For infant weight we are using Laica weighing scale model PS3001, whereas SECA adult weighing scale model 874 is used to assess maternal weight, we imported MUAC tape from UNICEF. SECA scale model 417 and SECA scale model 213 are used to measure infant length and maternal height respectively. The allowable difference between the two measurers according to study standard procedure for maternal MUAC is ± 0.5 cm, maternal weight is ±0.2Kg, maternal height is ±0.5cm, infant weight is ±20 gm, and ±0.4 cm for infant length, infant MUAC and infant head circumference. For longitudinal growth recording, the same anthropometry measurements are taken at day 27, 56, 85, 114, 143 and 179 of infant's life.

Primary outcome

The primary outcome of interest is length-velocity (cm/month) at 6 months. This will be measured through mean difference in length velocity measured at birth (or baseline) and at 6 months of age, expressed as change in cm/month.

Secondary outcomes

Other outcomes of interest are weight velocity (gm/kg/day), length-for-age z-score (LAZ), weight-for-age z-score (WAZ), and weight-for-length z-score (WLZ). Weight velocity (gm/kg/day) will be measured through mean difference in weight velocity measured at birth (or baseline) and at 6 months of age, expressed as change in gm/kg/day. Further, mean difference in specific z-score indicators (LAZ, WAZ and WLZ) measured at birth (or baseline) and at 6 months of age will be assessed. Further, anthropometry of mother is also assessed i.e. height (cm), weight (kg), and MUAC (cm), and mean change in each of these indicators between different arms will be assesses.

Biomarkers assessment (other secondary outcomes)

All laboratory specimens from mothers and infants will be collected on day 40 and 56 of infant's life i.e. two different time-point, defined based on administration oral Azithromycin to infants in intervention 2 at day 42. Therefore, the first time point (day 40) of specimen collection is pre-Azithromycin dose and

second time point (day 56) is after 14 days of Azithromycin dose. A window period of plus 7 days is allowed for specimen collection depending delay if any in Azithromycin dose to the infant. In order to create uniformity across the different arms, the same time points of specimen collection are applied to rest of the arms. Further, to complete the infant-mother dyad, same mother's specimens are also collected at the same time points. Difference in mean values (all continuous variable) of specimens collected at second time point (day 56) are of primary interest, and comparison will be made with values of specimens collected at first time point i.e. baseline measurement. Further, comparison will be made within the arm (second time point compared to baseline), between the arms as well as mother-infant dyad. Further, we will also calculate interquartile ranges for similar comparison as mention above.

As a part of laboratory procedures, infant blood will be collected from all comers where parents/caregiver are agreed for Hemoglobin (gm/dl), Ferritin (ng/ml), Transferrin (mg/dl), C-reactive protein (CRP) (mg/l), alpha1-acid glycoprotein (AGP) (mg/ml). Rationale for collecting Hemoglobin, Ferritin and transferrin is to assess if there is any difference in makers for iron deficiency anemia among infant in each arm. Hemoglobin assessment is being done with Hemacue equipment; while Ferritin and Transferrin will be performed using immune-turbidimetric assay on Roche Cobas c-311 automated clinical chemistry analyzer. AGP and CRP will be analyzed using the same assay and equipment to observe differences in these inflammatory biomarkers among three arms. In order to complement and link the findings of infant's biomarkers with maternal intervention, the 50 lactating women from each are also approached at same time point to collect the blood specimens for same biomarkers to complement the mother-infant dyad. These assays will be performed at Nutrition Research Laboratory (NRL) at Aga Khan University. Using the same subsample dyad, we are also planning to perform plasma proteomics to assess the element of antibiotic resistance.

Further, breast milk of same lactating women will be collected to assess quality of breast milk composition (macro- and micro-nutrients), Human Milk Oligosaccharides (HMO), immunoglobulins and microbiome analysis. The analysis of breastmilk specimens will be performed at Azad Lab, University of Manitoba. Material Transfer Agreement (MTA) will be developed with the University of Manitoba shipment of the specimens.

Among the same women and their infant i.e. 50 per arm, we will also collect stool specimens at same time points. We will assess inflammatory biomarkers in stool like Calprotectin (ug/gm), Lipokalin-2 (pg/ml), and Myeloperoxidase (MPO) using Elisa assay. Further, analysis of stool samples will also include detection of enteropathogens using TaqMan Array Card (TAC) system for Polymerase chain reaction (PCR), to be performed at Infectious Disease Research Lab (IDRL). Moreover, we will be performing targeted Bifidobacterium identification using real time PCR at IDRL. We will also send stool samples to University of Stanford after signing MTA, for further metagenomic work.

The specimens for further analysis and for future research (indefinite time) are stored at IDRL and NRL storage area at Aga Khan University at -80-degree Celsius freezers. The sample are de-identified with barcode, specific ID for different time point and mother-infant dyad, and color coded for different type of

specimen to create unique identification system. All the ethical aspects pertaining to storage of these samples are approved by Ethics Review Committee at Aga Khan University.

Monitoring and Quality assurance

There is a specific team from Aga Khan University with expertise in data management and trial implementation, working with the investigators, responsible for monitoring of general trial conduct such as auditing of trial data and processes to ensure completeness and accuracy of protocols, training of the research staff and outcome assessors. Further, there were independent experts who visited to monitor. Mechanism is also developed for the research teams to report out weekly progress on key progress indicators with the investigators at VITAL Pakistan Trust and Aga Khan University. Further, astringent quality assurance mechanism is developed through 10% of data is checked by trial supervisors and associate. All research teams are certified in Good Clinical Practice. Comprehensive training and refreshers are conducted on routinely basis. Teams involved in anthropometry are standardized by the WHO trained master trainers.

Data Safety and Monitoring Board

Independent group of experts, comprises of 5 members, formulated a DSMB for the trial are responsible for monitoring safety indicators, adverse events, and result of interim analysis. Interim analysis (blinded by arm) is scheduled when 50% of the enrollments complete their six months of follow-ups. Only DSMB members have access to the results of interim analysis, shared by independent statistician. Data of serious adverse events is shared with the board on a monthly basis in the form of progress report.

Participant safety

Close follow-up is done to ensure participant safety, and both the lactating women and infants are referred if needed to physician at the primary health care clinic, with facilitated referral to tertiary hospital when required. An independent DSMB monitors the safety of trial participants and provides trial oversight. Monthly reports are shared with the DSMB on serious adverse events with the authority that if a safety signal is observed, the DSMB may stop this trial prior to full recruitment. The safety net including facilitated referral and reporting is believed to minimize the chances of harm to participants.

Possible risks

There may be diarrhea, nausea, vomiting, skin rash, and abdominal distension after use of BEP. Similarly, infants may develop diarrhea, nausea, vomiting, skin rash, and abdominal distension post Azithromycin dose. We are systematically collecting information on all adverse events on each follow-up by asking about history of any illness since past visit and assessing danger signs on each visit. If mother and infant are identified with any suspected danger sign or symptom, there is a referral mechanism in place. A 24/7 phone number is provided where participant can call for any kind of illness and immediate referral is arranged. This information is well documented and recorded under adverse event reporting. For reporting purposes to ethics committee and DSMB, we specifically define ‘serious adverse events’ in two main

categories. First category is ‘fatal event” regardless of underlying cause, which occur among participants (mother and infant) during the follow-up period, and second category is any ‘non-fatal event’ where study participants (mother and infant) are end up with hospitalization or receive injectable therapy for any illness or diagnosis The risk management includes prevention through rigorous follow-ups, continuous monitoring of danger signs, documentation and prompt referrals. Every illness or danger sign reported or identified, is addressed through facilitated referral for both women and infants.**Statistical analysis**

This will be done on Stata version 15. Distribution of baseline characteristics across the arms will be done. The primary analysis will be intention-to-treat (ITT). We will compare mean length velocity (cm/month) (primary outcome), weight velocity (gm/kg/day), LAZ, WLZ, and WAZ in the two intervention arms with the control arm using repeated measures ANOVA adjusted for birth weight and age of infant at enrolment. If the outcome is missing for the intention to treat infants, means from that group will be imputed. Subgroup analysis will be done by maternal BMI and MUAC categories, and compliance with the intervention.

Participants and public involvement

Investigators has a very extensive experience of working with community and their representatives /elders. During protocol development phase, team discussed and took feedback on the research question and trial design from the community representatives. Furthermore, community perspective about the trial procedures, specially frequency and duration of follow-ups and biospecimens collection was also taken. Also, during the pilot phase the aim was not only to test the consent and questionnaires, but also to understand how community responds to different questions, and how sensitive information regarding antenatal and postnatal period can be collected in a more receptive and profound manner.

Discussion

This trial will provide evidence for the impact of nutrient supplementation in malnourished lactating women in low-and middle-income settings with high breastfeeding rates, for improvement of stunting and growth at six months of age. Dissemination and promotion of trial results will happen through small- and medium-scale communication events with stakeholders as well as symposia, publications, and via websites that will favor the exchange of ideas.

Strengths and limitations of this study

The study is uniquely place from the perspective that robust data on nutritional interventions are grossly lacking for malnourished mothers during periods of exclusive breastfeeding, especially for assessing an impact in combination with prophylactic dose of Azithromycin to the infant. Further, we foresee some potential biases in our trial, which may impose some limitations, they are listed below with countermeasures.

1. ***Selection Bias***: Selection bias can creep in due to the unblinded nature of the study. This has been mitigated through independent allocation sequence generation and block randomization in a sealed opaque envelope.
2. ***Performance bias and detection bias:*** Blinding of outcome assessors to the study arm through independent teams will decrease this bias.
3. **Incomplete outcome data (attrition bias):** The period of follow-up is 6 months, which substantially long and exposes the risk of lost to follow up. However, efforts are made to mitigate this through communication with the community in the trial run in period and rapport building with families. If any participant shifts out from the catchment area, if possible, they are followed at their new address till the outcome of the trial. Regardless, we will use all available data during analysis, even if missed at certain time point.
4. **Non-compliance bias:** Non-compliance could be due to the taste of the intervention and longer duration of consumption, which poses potential non-compliance with the intervention. This is addressed through detailed counseling at the time of consent and providing potential study participants 1 to 2 days for decision making and/or seeking input of household decision makers. Similarly, in the context of Pakistan, where prevalence of exclusive breastfeeding is between 35-40%, the continuous assurance by team that infants should be on exclusive breastfeeding for 6 months, is also very challenging. To address this, continuous counseling across the arm is the key, which is being done at the follow-ups.
5. **Contamination:** This is prevented by enrolling only one participant from a single household and also through provision of excess supplement as women are likely to feed the supplement to other members of the family.

Conclusion

The findings of the trial will help in reshaping the policies pertaining to prevention of undernutrition among lactating women and resultant poor growth of infants under 6 months of age. There will be significant policy implications for global problem of undernutrition among infants, for constrained health system with high burden of attributable infant deaths.

Trial status

Active protocol version number: 1.3, date January 16, 2019. Protocol amendments have been submitted, detail of protocol versions with date of amendment is provided in Table 2**.** Recruitment began in August 1, 2018. Currently, recruitment is ongoing which is expected to complete in June 2020, and last follow-up is expected to complete in November 2020. Database will be locked in January 2021.

Table 2 Protocol versions

Version	Date and changes
1.0	January 16, 2018 – Original protocol
1.1	July 22, 2018 – Introduction and background was improved
1.2	September 5, 2018 - Amendments on inclusion exclusion criteria
1.3	January 16, 2019 - More comprehensive plan of analysis was incorporated

Abbreviations

AGP	Alpha1-acid glycoprotein
BEP	Balanced energy-protein
BMI	Body mass index
CRP	C-reactive protein
CRF	Case report form
DSMB	Data Safety and Monitoring Board
ERC	Ethics Review Committee
HMO	Human milk oligosaccharides
IFA	Iron-folic acid
IRB	Institution Review Board
ITT	Intention-to-treat
IDRL	Infectious Disease Research Lab
LAZ	Length-for-age z-score
LW	Lactating women
LMIC	Low- and middle-income countries
MTA	Material Transfer Agreement
MPO	Myeloperoxidase
MMN	Multiple micronutrient
MUAC	Mid upper arm circumference
NRL	Nutrition Research Lab
PCR	Polymerase chain reaction
SQ-LNS	Small quantity Lipid-based Nutrient Supplements
TAC	TaqMan Array Card
WAZ	Weight-for-age z-score
WLZ	Weight-for-length z-score

Declarations

Competing interestsCompeting interests

The authors declare that they have no competing interests.

Funding

The trial is funded by Bill & Melinda Gates Foundation. Grant number OPP1179727. The sponsor has no role or ultimate authority over any of the trial related management, analysis, writing of the report; or the decision to submit the report for publication.

Ethics and consent to participate

Ethical approval of the trial was take from 'Institution Review Board (IRB)' of VITAL Pakistan Trust (Reference: 002-VPT-IRB-18 on April 3, 2018), 'Ethics Review Committee (ERC)' of Aga Khan University (Reference: 5234-Ped-ERC-18 on June 6, 2018), and 'National Bioethics Committee (NBC)' of Pakistan (Reference: 4-87/NBC-393/19/2170 on May 29, 2019). Written informed consent is voluntary taken from all participants and is available on request.

Availability of data and material

Processes will be developed to facilitate data sharing for scientific utilization in a collaborative manner. De-identified data with analytical/statistical codes will be available on the public domain 2 years after the publication of the main manuscripts with investigator support after approval of a proposal and with a signed data access agreement. It will be researchers whose proposed use of the data has been approved. Data is uploaded on the password secured cloud server.

Consent publication

Not applicable

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Authors contribution

AM: drafted the manuscript. YS, ATY, FJ, IN and AM: conceived and contributed to the design of the study, and developed study protocol and standard operating procedures. AM: implemented and supervised the study at the four sites. NY: coordinated the activities and contributed to the manuscript. BB: provided medical advice and support during the study. All authors provided feedback and approved the final manuscript.

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Ethics and consent to participate

Ethical approval of the trial was take from 'Institution Review Board (IRB)' of VITAL Pakistan Trust (Reference: 002-VPT-IRB-18 on April 3, 2018), 'Ethics Review Committee (ERC)' of Aga Khan University (Reference: 5234-Ped-ERC-18 on June 6, 2018), and 'National Bioethics Committee (NBC)' of Pakistan (Reference: 4-87/NBC-393/19/2170 on May 29, 2019). All participants who are enrolled, have been provided with written informed consent in the presence of a witness, either duly signed or thumb impression is provided by participant and the witness. Ethics committee has given approval for thumb impression of the participant is cannot read or write. Only designated study team is involved for obtaining the written informed consent. A copy of informed consent is provided to all participants attached in the file with study ID.

Availability of data and material

Not applicable

Consent publication

Not applicable

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Authors contribution

AM: drafted the manuscript. YS, ATY, FJ, IN and AM: conceived and contributed to the design of the study, and developed study protocol and standard operating procedures. AM: implemented and supervised the study at the four sites. NY: coordinated the activities and contributed to the manuscript. BB: provided medical advice and support during the study. All authors provided feedback and approved the final manuscript.

References

1. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, De Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. The lancet. 2013;382(9890):427-51.

2. Kerac M, Mwangome M, McGrath M, Haider R, Berkley JA. Management of acute malnutrition in infants aged under 6 months (MAMI): current issues and future directions in policy and research. Food and nutrition bulletin. 2015;36(1_suppl1):S30- S4.
3. Manohar H, Pravallika M, Kandasamy P, Chandrasekaran V, Rajkumar RP. Role of exclusive breastfeeding in conferring protection in children at-risk for autism spectrum disorder: results from a sibling case–control study. Journal of neurosciences in rural practice. 2018;9(1):132.
4. Alemayehu M, Argaw A, Geberemariam A. Factors Associated with Malnutrition among Lactating Women in Subsistence Farming Households from Dedo and Seqa-Chekorsa Districts, Jimma Zone, 2014. Dev. Ctry. Stud. 2015;5:114-22.
5. Musa S, Atena R. Malnutrition Markers And Nutritional Status In Qashqa'i Nomad Mothers. EJBS. 2013;17(1):70-5.
6. Haidar J, Muroki N, Omwega A, Ayana G. Malnutrition and iron deficiency anaemia in lactating women in urban slum communities from Addis Ababa, Ethiopia. East African medical journal. 2003;80(4):191-4.
7. Kiday H AM MG. Feeding practices, nutritional status and associated factors of lactating women in Samre Woreda, South Eastern Zone of Tigray, Ethiopia. Nutrition Journal. 2013;12(28).
8. Bailey RL WJK, Black RE. The epidemiology of global micronutrient deficiencies. Annals of Nutrition and Metabolism. 2015;66(2):22-3.
9. Abe SK BO, Ota E, Takahashi K, Mori R. Supplementation with multiple micronutrients for breastfeeding women for improving outcomes for the mother and baby. Cochrane Database of Systematic Reviews. 2016;2.
10. Kominiarek MA RP. Nutrition recommendations in pregnancy and lactation. Medical Clinics. 2016;100(6):1199-215.
11. Yaya S WR, Tang S, Ghose B. Intake of supplementary food during pregnancy and lactation and its association with child nutrition in Timor Leste. PeerJ. 2018;6:e5935.
12. Das JK LZ, Hoodbhoy Z, Salam RA. Nutrition for the next generation: older children and adolescents. Annals of Nutrition and Metabolism. 2018;72(3):47-55.
13. LH A. Multiple micronutrients in pregnancy and lactation: an overview. The American journal of clinical nutrition. 2005;81(5):1206S-12S.
14. Das JK LZ, Hoodbhoy Z, Salam RA. Nutrition for the next generation: older children and adolescents. Annals of Nutrition and Metabolism. 2018;72(3):47-55.
15. Adu-Afarwuah S, Lartey A, Okronipa H, Ashorn P, Peerson JM, Arimond M, et al. Small-quantity, lipid-based nutrient supplements provided to women during pregnancy and 6 mo postpartum and to their infants from 6 mo of age increase the mean attained length of 18-mo-old children in semi-urban Ghana: a randomized controlled trial, 2. The American journal of clinical nutrition. 2016;104(3):797-808.
16. Lanou H, Huybregts L, Roberfroid D, Nikièma L, Kouanda S, Van Camp J, et al. Prenatal nutrient supplementation and postnatal growth in a developing nation: an RCT. Pediatrics. 2014:peds. 2013-

850.
17. Katona P K-AJ. The interaction between nutrition and infection. Clinical Infectious Diseases. 2008;46(10):1582-8.
18. Lanou H, Huybregts L, Roberfroid D, Nikièma L, Kouanda S, Van Camp J, Kolsteren P. Prenatal nutrient supplementation and postnatal growth in a developing nation: an RCT. Pediatrics. 2014 Apr 1;133(4):e1001-8.
19. Christian P, Kim J, Mehra S, Shaikh S, Ali H, Shamim AA, Wu L, Klemm R, Labrique AB, West Jr KP. Effects of prenatal multiple micronutrient supplementation on growth and cognition through 2 y of age in rural Bangladesh: the JiVitA-3 trial. The American journal of clinical nutrition. 2016 Oct 1;104(4):1175-82.
20. Keenan JD, Bailey RL, West SK, Arzika AM, Hart J, Weaver J, et al. Azithromycin to Reduce Childhood Mortality in Sub- Saharan Africa. New England Journal of Medicine. 2018;378(17):1583-92.
21. O'Brien KS EP, Hooper PJ, Reingold AL, Dennis EG, Keenan JD, Lietman TM, Oldenburg CE. Antimicrobial resistance following mass azithromycin distribution for trachoma: a systematic review. The Lancet Infectious Diseases. 2018.
22. Robertson RC MA, Finlay BB, Prendergast AJ. The human microbiome and child growth–first 1000 days and beyond. Trends in microbiology. 2018.
23. Marques AH OCT, Roth C, Susser E, Bjørke-Monsen AL. . 2013 Jul 31;7:120. The influence of maternal prenatal and early childhood nutrition and maternal prenatal stress on offspring immune system development and neurodevelopmental disorders. Frontiers in neuroscience. 2013;7:120.
24. Somé JW AS, Jimenez EY, Hess SY, Ouédraogo ZP, Guissou RM, Vosti SA, Ouédraogo JB, Brown KH. Effect of zinc added to a daily small-quantity lipid-based nutrient supplement on diarrhoea, malaria, fever and respiratory infections in young children in rural Burkina Faso: a cluster-randomised trial. BMJ open. 2015;5(9):e007828.
25. Obanewa O NM. Maternal nutritional status during pregnancy and infant immune response to routine childhood vaccinations. Future virology. 2017;12(9):525-36.

Figures

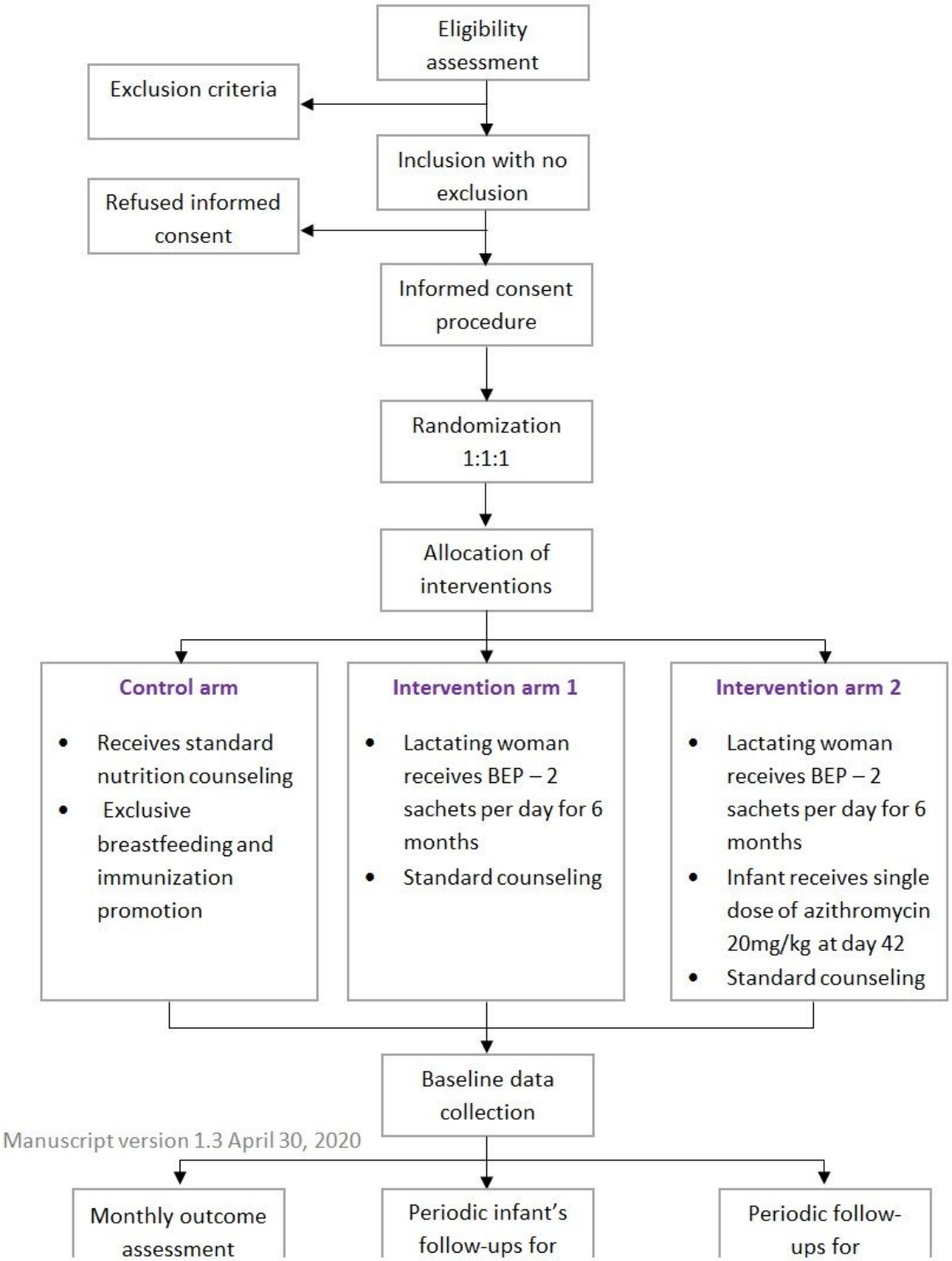

Figure 1

Consort flow diagram of trial

Timepoints	T_0[a]	Follow-ups				Outcome assessment	Specimen collection	Study endpoint
		Daily[b]	48 hourly	72 hourly	Weekly	Monthly		
		Day 1-13[c]	Day 14-25	Day 26-48	Day 49-179	Day 27, 56, 85, 114, and 143	Day 40-42 and 56+	Day 179
Enrollment								
Eligibility assessment	×							
Written informed consent	×							
Randomization and allocation	×							
Baseline data	×							
Intervention								
Nutrition and breastfeed counseling		×	×	×	×			
BEP distribution and compliance assessment[d]		×	×	×	×			
Azithromycin administration[e]					At day 42[f]			
Follow-ups								
24 hours breastfeeding recall		×	×	×	×			
Infant assessment for danger sign		×	×	×	×			
24 hours maternal food intake recall[g]					×			
Anthropometry						×		×
24 hours breastfeeding recall		×	×	×	×			
Infant assessment for danger sign		×	×	×	×			
24 hours maternal food intake recall[g]					×			
Anthropometry						×		×
Laboratory procedures							×	

[a] *Window period for eligibility assessment is 0-6 days of infant's life*
[b] *Follow-up start day is depending on age of the infant at the time of enrollment*
[c] *Age of the infant*
[d] *Only in intervention arm*
[e] *Only in intervention arm 2*
[f] *Window period of 7 days*
[g] *Only monthly basis*

Figure 2

Time schedule of enrollment, interventions, and assessments