PROTOCOL

Nutritional support for lactating women and Azithromycin for infants to improve growth outcomes in the peri-urban slums of Karachi, Pakistan – a Randomized Controlled Trial (Mumta LW Trial)

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1. INTRODUCTION

1.1. Background

Maternal undernutrition has a critical role in etiology of poor perinatal outcomes. (1) Low birth weight (LBW) babies being the most crucial among those with poor outcomes, contributes to 60-80% of all neonatal deaths, and (2) impacts nearly 20 million newborns. It is estimated that around 23.3 million newborns are also small for gestational age (SGA), {Lee, 2017 #3} (3) and nearly 18.6% of the global SGA births are attributed to maternal undernutrition during pregnancy. (4) In low middle income countries (LMIC), SGA alone contributes to 32 million cases of stunting. (5) Poor pre-pregnancy nutrition status (low body mass index), inadequate weight gain during pregnancy, as well as macronutrient and micronutrient deficiencies can lead to poor fetal growth, especially in low income settings. (6) Further, the poor nutritional status of these women, which also translates to undernutrition during the lactation phase, affects the nutritional status of newborns and infants. (7) Nearly 45% of infant deaths stem from poor nutritional gain during the early months of life and underlying wasting and stunting. (7) This also attributes to low quality of breastfeed in spite of exclusivity and impacts duration of breastfeeding resulting in malnourished infants even with exclusive breastfeeding.

In Pakistan, nearly 58% of households are food insecure or suffering from starvation. (8) Great disparities exist between urban-rural and within urban disadvantaged populations living in the poorest of slums. In Sindh province alone, 72% of households are food insecure and 50% are with moderate to severe hunger. (8) Around 18% of married woman of reproductive age in Pakistan are underweight based on body mass index (BMI) and also deficient of different micronutrients. For example, 41-42% of women are Vitamin A and Zinc deficient. (8) This directly impacts childhood stunting, wasting and underweight prevalence, which among under-five children in Pakistan is around 44%, 15% and 31%, respectively. (8) Furthermore, approximately 26% of babies have low birth weight and around 36% babies are SGA in Pakistan, (3) and these children are at the highest risk of developing moderate to severe stunting or wasting. This mandates the need for exploring the role of nutritional supplements in mothers on outcomes such as length velocity over the age of six months.

1.2. Review of Literature and trial rationale

1.1.1. Effect of nutritional supplementation during pregnancy and lactation on birth outcome and infant growth

To address the issue of malnutrition among pregnant and lactating women (PLW) and resultant malnutrition among infants, the World Health Organization Antenatal Care (WHO ANC) guidelines and National Guidelines for Community-based Management of Acute Malnutrition (CMAM) recommend the use of fortified balanced energy-protein supplements during pregnancy and lactation. Most importantly, the nutrient needs of infants during lactation depend primarily on the quality of breast milk which the infant is receiving from the mother, i.e. both the volume and composition of milk produced (quality of breast milk -macro- and micro-nutrients) are important. The quality of breast milk is very much linked to the mother's initial nutrient needs, nutritional intake, and nutritional status. Until recently, the WHO ANC guidelines had no recommendations on the use of these supplements in food insecure and undernourished settings, however, the new shift in policy provides an opportunity to generate evidence from developing countries to support the use of fortified balanced energy-protein supplements during the early lactation phase (0-6 months of infant's life) in order to see the impact on the infant's growth.

The ideal product would be one which is indigenously produced, fulfills dietary needs, fills energy/nutrient gaps, is palatable and is free of side effects. It is of importance that the supplement used shows benefit in averting post-neonatal outcomes and has a strong positive impact on growth and development. Additionally, there is also a strong debate on the impact of improved human gut microbiota (lactating mother and infant) on improved growth outcome. The type of diet consumed by the mother may have an impact on the gut microbiota of the infant, which may affect absorption of the macro- as well as micro-nutrients that the baby receives from the breast milk. Although the understanding of the microbiota concept is relatively new, current knowledge strongly suggests that the composition of microbiota has profound impact on the health status of humans, especially children. Diet plays a pivotal role in microbiota composition, and dietary types and patterns are linked with distinct combinations of bacteria in the intestine. Recently, the role of a chickpea based, balanced-energy protein supplement is under discussion, which may have great potential to improve gut microbiota. However, such supplements are newly available in Pakistan, with limited evidence available from the efficacy and effectiveness view point.

Available literature evaluating the evidence of Lipid-based Nutrient Supplements (LNS) in lactating women studied the efficacy of a small quantity of LNS (20 g/day with an energy of 118 kcal and protein of 2.6 g/day) provided during pregnancy, lactation and infancy and looked at the effect at 18 months of age. (13) A 6 months follow-up analysis of groups based on the intended supplement showed Mean ± SD of Length-

for-age z score of 0.62 ± 1.04, -0.78 ± 0.96, and -0.72 ± 1.03, for groups that were receiving daily Iron-folate (IFA) (pregnancy only), multiple micronutrient (MMN) (pregnancy and lactation), and LNS (pregnancy, lactation, and infancy) supplementation respectively. (13) Further, in a similar trial, LNS (20 g/day with energy of 118 kcal and protein of 2.6 g/day) was given to women during pregnancy and continued for 6 months after delivery, and the same infant LNS from 6 to 18 month of age with weaning diet. The hypothesis was that those infants who were in the LNS group will have a higher mean length at 18 months than the group where the mother received either iron or folic acid (IFA) during pregnancy only or Multiple Micronutrient (MMN) supplementation during pregnancy and lactation and the children who did not receive LNSs. This trial showed that at 18 months, the mean length showed an insignificant difference of 77.0, 76.9, and 76.8 cm (P value 0.90) in the IFA, MMN, and LNS groups respectively, and the prevalence of stunting was reported as 32.7%, 35.6%, and 37.9% (P value 0.54), respectively in all three arms. No intergroup differences were found in the mean weight, head circumference, or mid-upper arm circumference or the proportions with low z scores for these variables (P > 0.05). (14) Furthermore, another trial (Lanou H et al. 2014) looked at the impact of prenatal supplementation with LNS (72 gm/day with protein of 14.7 gm per sachet, 372 Kcal) compared to MMN at postnatal infant growth (until 1 year), there were no significant differences reported in linear growth velocity after birth. (15)

Justification for using Afzaaish Supplement

The new paradigm shift has strongly suggested the use of balanced protein energy supplementation (supplements in which protein provides less than 25% of the total energy content) to the undernourished. Use of such supplements by PLW may promote gestational weight gain, improve pregnancy outcomes and infant growth. (16) A variety of balanced energy-protein supplements are available, based on quality and type of protein, and its acceptability and feasibility for use that have shown varying effects. (17, 18). LNS used in different trials over the period of time are different from Afzaaish product proposed in this trial with respect to dose and composition. The supplement used in the Ghana study was designed specifically to provide micronutrients for a small amount of energy of about 118 kcal/day not really aiming to fill an energy gap, but rather to provide 4 macro minerals (Calcium, Potassium, Phosphorus and Magnesium) along with micronutrients and high quality protein of 2.6 gm/day in the amount of a 20 gm sachet per day. (13) The product was developed by International Lipid-Based Nutrient Supplements (iLiNS), following initial work in Malawi and Ghana. (19-21)

Until recently, in the context of Pakistan, experience with such ready-to-use formulation/supplements has been limited and in many cases the acceptability, efficacy and effectiveness of these products have not been properly studied to understand the contextual needs. Furthermore, many of the previously available

and tested products have low protein content, which has recently been recommended by policy actors. A product which provides approximately 16-20 grams of protein per day to PLW may produce a good impact on infants' growth outcomes. Even though there has been progress in the formulation of better nutrition supplements that are scalable for integration in MNCH and nutrition programs, evidence for their impact on birth outcomes and other postnatal growth effects on infants in LMIC is deficient. 'Afzaaish', a high protein, balanced protein-energy fortified ready-to-use supplement, is the main intervention in the proposed study. It is specifically designed for PLW to provide additional calories and it is locally produced in Pakistan. Afzaaish is a lipid-based and fortified nutrition supplement in the form of a semi-solid paste, with palatable taste which is likely to be accepted by the local population. Therefore, based on the need for evidence in the synthesis of new robust policies, this trial is envisioning the use of the 'Afzaaish' product among lactating women, which is high protein, balanced-energy, and has a chickpea base. This could impact the quality of breast milk and improve the nutritional status of mothers and infants, and may also have an impact in improving gut microbiota.

1.1.2. Effect of antibiotic prophylaxis for infant

Furthermore, there is also strong debate on the prophylactic use of antibiotics (especially the use of Azithromycin AZM) in averting the risk of stunting and underweight, although there is little evidence in support. The recent studies showed significant impact on the reduction of all causes of under-five mortality, but were unable to demonstrate a statistically significant difference in stunting, underweight, and low MUAC of children. The role of antibiotics on child growth and nutrition remains unclear, but the authors suggested larger studies and longitudinal trials may help determine any association. (9-12) The use and safety profile of AZM in infants is also well documented. A systematic review showed that AZM is the most common antibiotic used to treat several pediatric infections, including bronchopulmonary dysplasia (BPD). (22) Some side effects noted with its use include diarrhea, abdominal pain and vomiting. There is a potential side effect of infantile hypertrophic pyloric stenosis (HIPS). According to one author, the risk is high in the neonatal age and still needs to be further investigated. (23) Its use beyond the neonatal age is considered to be safe. (22, 23)

Justification for using Azithromycin

Literature has provided compelling evidence that AZM is safe for use in young infants and children (10, 23). Use of oral AZM on a mass level to control overall mortality in children living in rural regions has been found efficacious against respiratory diseases, diarrhea and malaria. (11, 12). In another study, giving a single oral prophylactic dose of AZM dramatically reduced the prevalence of infections in endemic areas.

(10) However, the treatment may have unintended consequences including possible adverse effects or resistance to antibiotics, but so far no data suggests resistance to a single oral prophylactic dose of AZM. (26) Furthermore, the mechanism of action of AZM is multidirectional and it plays a vital role in reducing inflammation because of its anti-inflammatory properties. (27, 28) Oral administration of AZM has also been found to improve the diversity of gut flora, improve immunity (29) and prevent the risk of infection among children. However, these theories have not been proven yet and further evidence to support prophylactic use of AZM among infants to promote infant growth is needed through large trials. Also, evidence from the combined effect of nutrition supplementation to lactating woman and prophylactic dose of AZM to infants is not yet available to see the overall impact on infants' growth outcomes.

1.2. Rationale for the trials

Robust evidence is needed via a field trial in the local context to evaluate the efficacy and effectiveness of the locally-produced, balanced energy-protein Afzaaish supplement alone or in combination with prophylaxis dose of AZM to infants on maternal and infant growth outcomes in low-income and food insecure settings. This could help to draw inferences for larger public health policy-making. This investment is specifically aiming to look at what impact a newly formulated nutritional supplement for pregnant and lactating women (PLW) can have on improving birth outcomes and as well as its potential to reduce wasting, stunting and underweight in infants.

2. PROJECT STRATEGY AND APPROACH

With the above background and rationale, we proposed a trial to address the impact of nutritional supplementation on lactating women by using a fortified, balanced energy-protein supplement alone in women or in combination with prophylactic use of azithromycin to infants. The trial will be conducted in peri-urban low-income areas of Karachi (Rehri Goth, Bhains Colony and Ali Akber Shah). The trial will be conducted in parallel to gain efficiency and obtain results on postnatal growth outcomes within a short timeline.

3. STUDY SETTING AND SITE DESCRIPTION

The trial will be conducted at Rehri Goth, Bhains Colony and Ali Akber Shah, Karachi. These are contiguously located impoverished peri-urban coastal slums with a population of approximately 250,000 residents based on the census conducted in 2017. These sites have an annual birth cohort of around 5000 each year. Fishing is the most common occupation (~30%). The population is multi-ethnic with Sindhi, Pashtun, Punjabi, Bengali, and Urdu-speaking groups. At our proposed site of work, a partner organization (Aga Khan University Hospital, AKUH) has a long-standing demographic surveillance system (DSS) in place. This includes 2 monthly Married Women Surveillance Registration (MWSR) rounds, which includes all women of 13- 49 years of age living within the designated area. During the surveillance rounds, the vitals of the women, including mortality, pregnancy status, and movement in and out the area, as well as the status of under 5 children, are recorded.

4. OBJECTIVES

4.1. Primary objective

a) To assess the efficacy of a fortified, balanced energy-protein supplementation intake using a ready-to-use product for at least 6 months during lactation and exclusive breastfeeding, in improving length velocity as the primary outcome.

b) To assess the efficacy of a fortified, balanced energy-protein supplementation intake using a ready-to-use product for at least 6 months during lactation and exclusive breastfeeding along with oral Azithromycin at 42 days of age to the baby as a single dose in improving length velocity as the primary outcome.

4.2. Secondary objective

a) To assess the efficacy of a fortified, balanced energy-protein supplementation intake using a ready-to-use product for at least 6 months during lactation and exclusive breastfeeding in improving breast milk composition over the period of six months and weight velocity, length-for-age, weight-for-length, and weight-for-age Z scores as secondary outcome at 6 months of age as compared to the standard of care.

b) To assess the efficacy of a fortified, balanced energy-protein supplementation intake using a ready-to-use product for at least 6 months during lactation and exclusive breastfeeding in improving breast milk composition over the period of six months along with oral Azithromycin at 42 days of age to the baby as a single dose, and weight velocity, length-for-age, weight-for-length, and weight-for-age Z scores as secondary outcome at 6 months of age as compared to the standard of care.

5. HYPOTHESES

Length velocity at 6 months of infant's age

H_a = Mean difference in length velocity of >0.12cm/month to look at comparisons between multiple arms

H_o = Mean difference in length velocity of ≤ 0.12cm/month to look at comparisons between multiple arms

Growth velocity at 6 months of infant's age

H_a = Mean difference in growth velocity of >0.4 gram/kg/day to look at comparisons between multiple arms

H_o = Mean difference in growth velocity of ≤ 0.4 gram/kg/day to look at comparisons between multiple arms

Length-for-Age Z-score (LAZ) at 6 months of infant's age

H_a = Mean difference in LAZM >0.5 to look at comparisons between multiple arms

H_o = Mean difference in LAZM ≤0.5 to look at comparisons between multiple arms

Weight-for-Length Z-score (WLZ) at 6 months of infant's age

H_a = Mean difference in WLZ >0.5 to look at comparisons between multiple arms

H_o = Mean difference in WAZM ≤0.5 to look at comparisons between multiple arms

Weight-for-Age Z-score of (WAZ) at 6 months of infant's age

H_a = Mean difference in WAZM >0.5 to look at comparisons between multiple arms

H_o = Mean difference in WAZM ≤0.5 to look at comparisons between multiple arms

6. METHODOLOGY

6.1. Study Design

A randomized, controlled, open-label, community based trial to assess the impact of the provision of nutritional intervention to lactating women from 0-6 months post birth outcome and one prophylactic dose of AZM to infants on the infants' growth outcome. Individuals responsible for outcome assessment (the monthly anthropometry) will be kept blinded from the participants' information randomized in each arm.

6.2. Study Population

Lactating women and their babies until 6 months of age, residing in low-resource settings

6.3. Inclusion and exclusion criteria

Inclusion Criteria

1. Live birth, captured within 0-6 days
2. MUAC of mother < 23.0 cm
3. Permanent resident of the catchment area, i.e. resident since last the 6 month at least (not temporary migrant or guest/relative)
4. Confirmed intention to exclusively breastfeed their child for at least 6 months of age
5. Child continues to stay with mother for the next six months
6. Provision of written informed consent

Exclusion Criteria

1. Working and employed lactating women staying away from the baby and the house, as exclusive breastfeeding is a prerequisite for enrollment in this trial
2. VLBW i.e. < 1500 gram
3. A mother who is unable to breastfeed the child due to certain medical conditions.
4. Child with congenital abnormalities (example: Cleft palate/lip, hydrocephalus, microcephalus, intestinal obstruction, etc.) or other serious illness at the time of enrollment, which was identified by the study physician (suspected sepsis for example)
5. Previously enrolled in this trial or PW trial
6. Known allergy to food items like, peanut, lentils, milk etc.

6.4. Interventions

	Arm A (Control)	Arm B (Afzaaish Only)	Arm C (Afzaaish + AZ)
Afzaaish Supplement	Not Applicable	Two sachets per day from the day of enrollment to 6 months A weekly supply of sachets will be given to each LW	Two sachets per day from the day of enrollment to 6 months A weekly supply of sachets will be given to each LW
Azithromycin	Not Applicable	Not Applicable	To be given to infants of LW Infant dose: 20mg/kg at 42 days of age after birth
Standard Counseling	As mentioned below (5.4.3)	As mentioned below (5.4.3)	As mentioned below (5.4.3)

6.4.1. Nutritional Supplement

'Afzaaish' is the main intervention in the proposed study. Locally produced in Pakistan, it is specifically designed for PW and LW to provide additional calories. Afzaaish is a fortified, semi-solid, lipid-based nutrition supplement which is available as a ready-to-use paste. As per information provided by the manufacturer, table 1 provides the product specifications:

Table 1: Afzaaish Supplement Product Specifications

S. No.	Ingredients	% Dry Weight
1	Roasted chickpeas	21.2
2	Skimmed milk powder	20
3	Soybean oil	15
4	Roasted peanuts	13
5	Sugar	10
6	Palmolein oil	9
7	Roasted yellow lentils	4
8	Premix – vitamin and minerals (approximate premix incorporation rate = 34 per metric ton)	3.4
9	Hydrogenated vegetable fat as stabilizer	2
10	Maltodextrin (14-18 dextrose equivalent)	2
11	Emulsifier	0.34
12	Antioxidant	0.068

According to the product information provided by the local manufacturer, the detailed range of nutrients and nutritional values per 100 gram of the finished product is mentioned below (these ranges are provided by the manufacturer and mentioned on the label of the product):

i. Energy = 510-560 Kcal

ii. Protein = 11-16 gram
iii. Skimmed milk powder protein = 3.6 grams
iv. Fat = 26-36 grams
v. Omega 3 fatty acids = 1.80 grams
vi. Omega 6 fatty acids = 6.10 grams
vii. Added sugars = 10 grams

Serving size is 75 grams according to the label. The product has a shelf life of 18 months with respect to the climate of Pakistan and 24 months when stored up to 30^0 C at 65% relative humidity. Detailed product specification with recommended daily requirement is provided in table 2 below:

Table 2: Afzaaish Supplement- Recommended Daily Requirement

IOM DRIs/RDAs/AIs and FAO/WHO RNIs for Pregnant and Lactating Women -Macronutrients and Micronutrients							Micronutrients - Minimum and Maximum by the expert convening		Afzaaish product - Locally produced ready-to-use supplement			
IOM Energy DRIs for 19-30 years			FAO/WHO RNIs for 19-30 years									
Pregnant women (additional kcal/day)									Energy (Kcal)			
First Trimester	Second Trimester	Third Trimester	First Trimester	Second Trimester	Third Trimester		Mini m-um / Target (EAR)	Maxim-um (RDA)	Min per 100 gm	Label per 100 gm	Label per 75 gm sachet (One serving minimum)	Expected energy that dose of 2 sachets per enrolled PW/LW will provide (150 gm per day) (Approx.)
None	340	452	85.0	285.0	475		Total energy 250	Total energy 500	510	510-560	400	800
Lactating women (additional kcal/day)									Macronutrients and Micronutrients			
Less than 6 months of infant age												
330			505									
Requirement		Pregnant	Lactating	Pregnant	Lactating		Maximum 10% of total energy					
Carbohydrate (g)	RDA	175	210									
Protein (g)	RDA	71	71	Additional 1, 9 and 31 g in 1st, 2nd and 3rd trimester	Range of additional 14.3 to 16.2 g in 1st 6months		14	18	11	11-16	10.5	21
Lipid total (g)	-	-	-				10% of total energy	60% of total energy	26	24-36	24	48
Linoleic acid (g)	AI	13.0	13.0						2.6	2.6-6.10	2.6	5.2
Linolenic acid (g	AI	1.4	1.3				1.3		0.3	0.3-1.8	0.3	0.6
Fiber (g)	AI	28	29									
Vitamin A (µg RE)	RDA	770	1300	RNI:370 Safe intake:800	RNI: 50, Safe in take:850		550	770	550	550	4 2	24
Vitamin D (µg)	RDA		5	15	15		10	15	1	15	11 2	22.4
Vitamin E (mg)	RDA	15	9	NR	NR		16	19	16	16	12	24
Vitamin K (µg)	AI	90	90	55	55		72	90	27	27	20.2	40.4
Thiamin (mg)	RDA	1.	1.4	RNI:1.4	RNI:1.5		1.2	1.4	1	1	0.75	1.5
Riboflavin (mg)	RDA	1.4	1	1.4			1.3	1.6	2	2.1	1.57	3.14

			6		.6			1			
Niacin (mg)	DA	1	1	18	17	14	18	3	13	9.75	19.5
Vitamin B6 (mg)	RDA	1.9	2.0	1.9	.0	1.7	2	1.8	1.8	1.35	2.7
Folate (µg)	DA	600	500	EAR:520, RNI:600	EAR:450, R NI:500	400	600	33	330	27	494
Vitamin B12 (µg)	RDA	2.6	2.8	EAR:2. , RNI:2.6	EAR:2.4 RNI:2.8	2.4	2.8	2.	27	2	4
Vitamin C (mg)	RDA	85	120	55	70	100	120	60	6	45	90
Calcium (mg)	RDA	1000	1000	1200 (Last Trimester)	000	500	1000	535	535	400	800
Iron (mg)	RDA	27	29		10 to 30	22	27	10	10	7.5	15
Zinc (mg)	RD	11	12	5.5, 7, 10 (1st, 2nd 3rd trimester)	9.5, 8.8 (0-3, 3-6 months)	15	20	11	11	8.2	16.4
Iodine (µg)	RDA	220	20	200	200	209	290	00	100	75	150
Biotin (µg)	AI	30	35	30	35	28	35	60	60	5	90
Pantothenic acid (mg)	AI	6	7	6	7	5.6	7	4	4	3	6
Choline (mg)	AI	450	550			220	550				0
Phosphorus (mg)	RDA	700	700			300	700	450	450	337	674
Magnesium (mg)	RDA	350	310	220 for females> 19, NR	220 for females> 19, NR	145	350	150	150	112	224
Manganese (mg)	RDA	2.6	2.6			2.1	2.6	1.2	1.2	0.9	1.8
Copper (µg)	RDA	1000	1300			1	1.3	140 0	1000	1000	2000
Selenium-um (µg)	RDA	60	70	28 (2nd trimester) 30 (3rd trimester)	35 (0-6 months post-partum)	60	70	20	20	15	30
Potassium-um (g)	AI	4.7	5.1			2	5.1	0.9	0.9	.675	1.35

IOM = Institute of Medicine
FAO = Food and Agriculture Organization of the United Nations
WHO =World Health Organization
DRI = Dietary Reference Intake
AI = Adequate intake
EAR = Estimated Average Requirements
RDA = Recommended Dietary Allowance
RNI = Reference Nutrient Intake

6.4.2. Antibiotic Azithromycin

Azithromycin is an azalide antibiotic with a good safety profile and it can be safely used in infants. Azithromycin will be given to infants at 42 days of age after birth at a standard dose of 20mg/kg as a single oral dose in the form of an oral suspension. Use of oral Azithromycin can be administered to neonates as a single dose of 20mg/kg without the report of any adverse events and is thus considered safe. (23)

6.4.3. Standard Counseling and Referral System for Lactating Women

Once LWs are confirmed to be within the eligibility criteria, they will be given routine ANC counseling, as well as nutritional and exclusive breastfeeding counseling, immunization services, primary health care services for newborn/infants and referrals in case of the newborn/infant when and where required. Standard nutritional and exclusive breastfeeding counseling is an essential component of the WHO MNCH program. The strategy focuses on:

i. Initiation of breastfeeding within the first hour of life
ii. Exclusive breastfeeding – that is, the infant only receives breast milk without any additional food or drink, not even water
iii. Breastfeeding on demand – that is, as often as the child wants, day and night
iv. No use of bottles, teats or pacifiers
v. Promoting a healthy diet by increasing the diversity and amount of foods consumed
vi. Promoting adequate weight gain through sufficient and balanced protein and energy intake, through the consumption of a variety of foods, including green vegetables, meat, liver, fish, beans, lentils, dairy products and fruits.

Apart from counseling and follow-ups, LW in Arm A will be provided a small package once at the time of enrollment in good faith for contributing their time for this trial. Furthermore, nutritional counseling and provision of high quality standard of care will be the same for all arms, as per WHO guidelines. Along with this, all groups will be routinely followed for assessing danger signs and to check the commitment with continuation of exclusive breastfeeding habits.

6.5. Enrollment of Lactating Women

LW will be enrolled in the project based on case definition (inclusion criteria) and eligibility assessment. The research team will identify potentially eligible candidates during pregnancy and wait till the birth outcome to visit the household within 0-6 days of live birth to confirm eligibility. When the birth outcome is reported, following eligibility assessments, LWs will be enrolled in the trial after all key procedures of consent and randomization. Written voluntary informed consent in the presence of a witness will be taken

to ensure voluntary participation in the trial. After consent, LW will be randomized following the defined sampling strategy.

Related to notification of birth outcomes, VPT has a very robust surveillance system through which community health workers will notify the birth outcomes in the existing system through household PW follow-ups designed for this purpose. Moreover, for this trial to create liaison with the community, a very successful phone call system has been established through which the community is connected with a designated health worker 24/7. Therefore, any woman who delivers at any other facility or at home can also notify the study team though a phone call. An incentive has been established to encourage the family members to call within the time limit.

Once an LW with MUAC <23.0 cm and birth outcome captured within 0-6 days is identified, the LW will be counseled with regard to the trial procedure and will be given a time of 24-48 hours to discuss it with family members. Where required, randomization teams will visit the household to develop liaison with the family and explain the procedure. Once the woman and family demonstrate voluntary willingness, informed consent will be explained once again before the final signing of the consent in the presence of a witness. Following this, she will be randomized in one of the three arms according to the trial strategy.

6.6. Informed consent procedure for trial recruitment

LW fulfilling the case definition and inclusion criteria are required to sign informed consent voluntarily to participate in the trial. A verbal consent (informal permission to develop rapport) of the husband or any other household member will also be sought in order to avoid dropping out of the trial at a later stage. Consent for study participation will be obtained by the research assistant in the presence of a community health worker and will involve detailed verbal communication in the study participants' native language to ensure comprehension of the trial and study procedures. Illiterate guardians will be requested for thumb impression while literate guardians will be requested to sign the consent form. All the enrolled participants in all of the groups will get a copy of the consent form duly signed by the study officials. The informed consent procedure will be conducted in the local languages.

6.7. Randomization and Allocation Concealment

The block randomization technique will be used in this trial and works by randomizing participants within blocks such that equal numbers are assigned to each treatment. Blocks will be of varying size as 3, 6, and 9 in a ratio of 1:1:1. In each arm, 319 LWs will be randomized. The randomization list will be generated independently by a data management unit not involved in recruitment, outcome or any other aspect of the study. Following the list, the sealed envelopes will be prepared. All those LWs who consent to participate in the study will be randomized into the respective arm by the randomization team. For

randomization in Afzaaish LW trial, the sealed envelope technique will be used. Envelopes will be placed in a box file that will be carried by the randomization team. After taking consent from the LW, the research staff will pick the top most envelope from the file in a sequential manner. This allows them to follow the implemented strategy of 'sequence within a block' as well as 'block sequence'. The opening of an envelope will reveal the unique randomization ID with the allotted treatment arm the LW would be enrolled in.

6.8. Blinding of Outcome Assessor

There will be two separate assessment teams involved in this trial. Outcome assessment team (Anthro team) will include the research staff that will be responsible for conducting monthly assessments of the outcomes. This team will remain blinded with respect to the allocation of interventions and will be restricted with their inquiries. Their work will be limited to anthropometry and outcome assessments according to the designed schedule.

Team 2 (Follow-up Team) will comprise of the research staff who will be responsible for compliance checks and distribution of Afzaaish sachets on a routine basis, as well as for administration of the Azithromycin dose to the infants and food frequency checks. Technically, this team cannot be blinded to the intervention allocation. Also, they will be involved in conducting regular counseling sessions on exclusive breastfeeding and healthy diet. This team will also be restricted to follow the standard counseling protocol for all the enrolled LW in the trial.

6.9. Study Drug Storage, Dispensing and Administration

Afzaaish Supplement

LWs allocated to arms B and C will receive the intervention supplement `Afzaaish' soon after enrollment. Each enrolled LW in this arm will receive a routine supply of Afzaaish sachets (daily or weekly, depending on follow-up schedule). Sachet will be provided by trained research staff, either at the clinic or at the LW's home depending on nature of follow-up visit. LWs in this arm will be counseled to consume two sachets in a 24-hour period (2 sachet per day). The sachets will be provided in routine installments until the child reaches the age of 6 months. On every visit, the LW will return the empty sachets and in exchange will receive new ones.

Azithromycin

Azithromycin will be given to infants at 42 days of age of LW randomized in Arm C and administered orally as a single dose using 20mg/kg as a standard for dosing. The suspension will be reconstituted by the trained staff and the dose will be calculated according to the infant's weight, before administering to the subject. For dose accuracy 1 bottle of Azithromycin will be reconstituted and used for each infant, while the leftover dose will be archived for future reference purpose.

6.10. Assessment and Recording Data on Study Participants

The number of times each participant will be contacted for outcome assessment is summarized below (Table 4):

1. Maternal and infant anthropometry at birth (baseline, within 0-6 days of birth) and then on monthly basis until 6 months of age.
2. Every month, 24 hours food recall data will be collected from every enrolled LW

6.11. Laboratory sampling for study purpose only

a) Breast milk samples on 50 randomly selected women from each arm will be collected for future assessment of micronutrients, macronutrients and microbiome. Aseptic hand expression technique will be followed for collection of breast milk for microbiome analysis (for Biobank for further analysis in future)

b) Stool specimens will be collected from the same 50 randomly selected LWs and their infants from each arm for TaqMan Array Card (TAC) and Myeloperoxidase (MPO) using metagenomics approach (for Biobank for further analysis in future)

c) Blood specimen will also be collected from the same 50 randomly selected LWs for the purpose of checking hemoglobin levels, serum ferritin, transferrin receptor and acute phase proteins such as AGP and CRP and for Biobank for further analysis in future for proteomics.

d) Infant anemia, hemoglobin, serum ferritin, transferrin receptor and acute phase proteins such as AGP and CRP and for Biobank for further analysis in future for proteomics (for all enrolled subjects upon consent from parents).

6.12. Follow ups

There will be specific assessment teams involved in this trial who will conduct their specific follow ups as per the below mentioned schedule to assess the newborn periodically and also measure the compliance of Afzaaish (Arm B and C) also providing routine counseling to all participants (all arms). Furthermore, the follow-up team will comprise of research staff that will be responsible for monthly 24 hour food recall checks. Additionally, they will also be responsible for assessing exclusive breastfeeding status, i.e. exclusive, predominant or partially, in all arms. 24 hours exclusive breastfeeding recall related data will be collected on the visit days. Child illness related history, care seeking, hospitalization and medication related history will be recorded at each visit. These follow up teams will also be in charge of household health promotion and education counseling for all the arms following the already established standard care counseling procedures. Exclusive breastfeeding will be assiduously promoted and precautionary counseling will be done regarding risks associated with giving the infant solids or liquids other than breast milk.

Also an outcome assessment team (Anthropometry team) will be responsible to follow babies on a monthly basis until the age of 6 months to perform anthropometry on both LW and infants. Schedule for follow-ups are as follows:

Activity	**Follow-up days, according to the age of the child after enrollment**		
1. Afzaaish Compliance, newborn assessment (WHO algorithm of danger sign), past illness history, hospitalization related history, other care seeking, medication related history, 24 hour EBF recall, Afzaaish compliance -44 days are meant for pre and post Azithromycin dose)	1. 1 2. 2 3. 3 4. 4 5. 5 6. 6 7. 7 8. 8 9. 9 10. 10 11. 11 12. 12 13. 13 14. 15 15. 17 16. 19 17. 21	18. 23 19. 25 20. 29 21. 32 22. 35 23. 38 24. 41 25. 42 26. 43 27. 44 28. 48 29. 52 30. 59 31. 67 32. 74 33. 81 34. 88	35. 95 36. 102 37. 109 38. 116 39. 123 40. 130 41. 137 42. 144 43. 151 44. 158 45. 165 46. 172 47. 179
2. 24 hours food recall	1. 7 2. 35 3. 67 4. 95 5. 123 6. 151		
3. Anthropometry and vaccination record	4. 27 5. 56 6. 85 7. 114 8. 143 9. 177		

7. OUTCOME ASSESSMENT

7.1. Primary Outcome

a. **Length velocity at 6 months of infant's age**

Mean difference in length velocity of >0.12cm/month to look at comparisons between multiple arms

7.2. Secondary Outcomes

a. **Growth velocity at 6 months of infant's age**

Mean difference in growth velocity of >0.4 gram/kg/day to look at comparisons between multiple arms

b. **Length-for-Age Z-score (LAZ) at 6 months of infant's age**

Mean difference in LAZM >0.5 to look at comparisons between multiple arms

c. **Weight-for-Length Z-score (WLZ) at 6 months of infant's age**

Mean difference in WLZ >0.5 to look at comparisons between multiple arms

d. **Weight-for-Age Z-score of (WAZ) at 6 months of infant's age**

Mean difference in WAZM >0.5 to look at comparisons between multiple arms

Other Pre-specified Outcomes

a. **Breast milk composition of 50 randomly selected LWs**

To assess the quality of breast milk (macro and micronutrients) and microbiome analysis

b. **Stool specimens of 50 randomly selected LWs and Infants**

Stool will be collected for TaqMan Array Card (TAC) analysis of enteric pathogens and Myeloperoxidase (MPO) as a marker of gut inflammation and will be assessed using a metagenomics approach.

c. **Blood Specimens of 50 randomly selected LWs and all Infants enrolled in the trial**

To assess hemoglobin, serum ferritin, transferrin receptor and acute phase proteins such as AGP and CRP.

7.3. Trial procedures for outcome assessment

Assessments/ Specimen	Arm	At enrolment	On each visit till age of 6 months	Monthly till age of 6 months	At 40-42 day of infants age (Before AZM dose)	At 56 day of infants age	At the age of 6 months (study endpoint)
Assessments at field							
Afzaaish Sachet distribution and Compliance	B and C	✓	✓				
Azithromycin	C				✓ At 42 days		
Vital status	A, B, C	✓	✓				
Standard nutrition and health promotion counseling	A, B, C	✓	✓				
Infant anthropometry	A, B, C	✓		✓			✓
Maternal anthropometry	A, B, C	✓		✓			✓
24 hours Food Recall	A, B, C	✓		✓			✓
Lab investigations for study purpose (all Infants, where family is agree)							
Hemoglobin	A, B, C				✓	✓	
Serum Ferritin	A, B, C				✓	✓	
Transferrin receptors	A, B, C				✓	✓	
AGP	A, B, C				✓	✓	
CRP	A, B, C				✓	✓	
Proteomics (Biobank)					✓	✓	
Stool specimens (50 infants only)	A, B, C				✓	✓	
Lab investigations for Bio bank purpose only (50 LWs only)							
Breast milk	A, B, C				✓	✓	
Stool specimens	A, B, C				✓	✓	
Hemoglobin	A, B, C				✓	✓	
Serum Ferritin	A, B, C				✓	✓	
Transferrin receptors	A, B, C				✓	✓	
AGP	A, B, C				✓	✓	
CRP	A, B, C				✓	✓	
Proteomics (Biobank)	A, B, C				✓	✓	

8. ADVERSE OUTCOMES

8.1. Afzaaish supplement related – lactating woman only

The trial participants will be instructed/counseled to report any adverse events (minor or major), which may be serious or non-serious in nature. The reporting of the adverse event will take into account any event which may be due to the use of nutritional supplement or with the use of any adjunct therapy, or even death of the trial registered participant (due to any circumstance). *Nutravigilance* is the term used for assessment, understanding, reporting, and prevention of any kind of adverse effects related to the use of food or nutritional supplement. The reporting system for adverse event for dietary and nutritional supplement will follow the Food and Drug Administration (FDA) guidelines so that the information gathered from LW can be used for generating signals/alerts for possible public health concerns and subsequently assessing those signals and taking appropriate safety actions since this nutritional supplement used by LW will ultimately be associated with the health and outcomes of the infants.

Reporting of adverse events on nutritional and dietary supplement includes the range of events from nausea, vomiting, diarrhea, abdominal distension, abdominal pain and rash. LW will be counseled to immediately report any serious condition which appears during the enrollment period, either during follow-up visits by research team or any time through a call on the number provided to them. For this trial the data collected on adverse events and other related reports will be provided by Principal Investigator directly to the DSMB on a monthly basis (serious) in the form of a report, while any fatal adverse events will be reported to DSMB and AKU/VPT IRB within 72 hours of event reported. On every follow-up visit, the research team will collect information for any potential side-effect and health status of LW through active and vigilant surveillance.

Adverse and serious adverse event reporting and categorization

The following **adverse events** will be observed for and documented:

1. Vomiting: The forceful expulsion of gastric contents reported by LW.
2. Diarrhea: Diarrhea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the LW), reported by LW.
3. Abdominal distention or pain: Conditions/symptoms reported by LW as feeling full, tight, or swollen in the abdomen, i.e. abdomen reported as swollen (distended), hard, and painful, reported by LW.
4. Rash: An appearance of itchy, red, painful, and irritated skin, visible to health worker or reported by LW.

5. Any other serious symptom: If LW has reported any unusual symptoms, which she has never experienced before.

Documentation of adverse event

Adverse event reporting form will be filled against every event which is reported during the course of the study period and in case of any death the routine verbal autopsy form will also be filled. Based on the information provided in the forms, the decision will be made on whether a serious or non-serious adverse event is related to trial intervention.

Serious Adverse Event

Any adverse event will be labeled as a "Serious Adverse Event" (SAE) if it leads to:

a. Intravenous therapy
b. Hospitalization
c. Life threatening situation
d. Death

Narrative description of serious adverse event will be provided in a document template and attached into the participants file. After reporting of any suspected adverse event, the senior research team/leader, including the study physicians will define the linkages of event with Afzaaish and fill an adverse event reporting form which will define the event in relation to Afzaaish as:

a) Not related
b) Unlikely related
c) Possibly related
d) Definitely related
e) Not assessable

8.2. Azithromycin related – Infant only

To establish safety profile for AZM prophylaxis dose, the infant will be monitored for the next 48 hours. After administration, data will be collected and any side-effects (observed or reported) will be noted by the research team. They will revisit the infants after 24 hours (i.e. after dose, two consecutive visits will be performed with a 24 hour gap). For this trial the data collected on adverse events and other related reports will be provided by Principal Investigator directly to the DSMB on a monthly basis (serious) in the form of report, while any fatal adverse events will be reported to DSMB and AKU/VPT IRB within 72 hours of event reported.

Adverse event reporting form will be filled against every event which is reported over the course of study period and in case of any death the routine verbal autopsy form will also be filled. Based on the information provided in the forms, decision will be made on whether to classify the event as serious or non-serious and also if it was related to trial intervention or not. Post-dose (43 and 44 day) visits will be performed to monitor and observe any suspected adverse event in the infants which are most likely to appear after AZM administration. If in any case, the dose is delayed, the 2 consecutive visits days will be changed accordingly.

Adverse and serious adverse event reporting and categorization

Based on the literature (30), following potential adverse event in infants related to AZM will be monitored:

i. Gastrointestinal:
    a. Vomiting: Persistent vomiting or projectile vomiting in an infant reported by mother, within 24 hours of dose administration (Observed or reported).
    b. Poor feeding/suck: If an infant develops poor feeding/suck or is unable to feed within 24 hours of dose administration (Observed or reported).
    c. Necrotizing Enterocolitis: Abdominal distention/tenderness, bowel movements, signs of infection such as apnea (stop breathing), and lethargy within 24 hours of dose administration in an infant (Observed or reported).
    d. Diarrhea: Loose stool in an infant reported by mother, lead to severe dehydration, within 24 hours of dose administration (Observed or reported).

ii. Respiratory:
    a. Respiratory distress: Fast breathing (60 or more breathes per minutes) and/or severe chest in drawing and/or baby turn bluish, within 24 hours of dose administration (Observed or reported).

iii. Rash: An appearance of itchy, red, painful, and irritated skin, visible by health worker or reported by family, within 24 hours of dose administration.

Documentation of adverse event

Adverse event reporting form will be filled against every event which is reported during the course of the study period and in case of any death the routine verbal autopsy form will also be filled. Based on the information provided in the forms, the decision will be made on whether a serious or non-serious adverse event is related to trial intervention.

Serious Adverse Event

Any adverse event will be labeled as a "Serious Adverse Event" (SAE) if it leads to:

a. Intravenous therapy
b. Hospitalization
c. Life threatening situation
d. Death

Narrative description of serious adverse event will be provided in a document template and attached in the participants file. After reporting of any suspected adverse event, the senior research team/leader, including the study physicians will define the linkages of event with AZM and fill an adverse event reporting form which will define the event in relation to AZM as:

a) Not related
b) Unlikely related
c) Possibly related
d) Definitely related
e) Not assessable

9. COMPLIANCE WITH AFZAAISH SUPPLEMENT AND AZITHROMYCIN

Although, every woman enrolled and randomized, will be included in intention to treat analysis, however for the purpose of trial administration and assurance of maximum compliance, the following criteria will be followed to define compliance:

a) Team will be tasked to ensure at least 75% compliance in each month with the consumption of a supplement which will be given to LW enrolled in Arm B and C

Compliance (for 6 months)	100% Compliance in each month	75% Compliance in each month	Low compliance and concern for counseling
Afzaaish related- 2 sachets of Afzaaish to be consumed in 24 hours	If 60-65 sachets consumed in 30 days	If 45-49 sachets consumed in 30 days	LW who has consumed <45 sachets for 2 consecutive months (based on each month's compliance)

b) Team will ensure that dose of Azithromycin was administered on 42 days (window period of 7 days).

c) Exclusive Breastfeeding: Team will be tasked to achieve at least 75% compliance each month in all arms

Compliance (for 6 months)	100% Compliance in each month	75% Compliance in each month	Low compliance and concern for counseling
Only breastfeeding from LW enrolled in the trial, with no other things to drink or eat given to child	Only breastfeeding from LW enrolled in the trial, with no other things to drink or eat given to child, confirmed on each visit	If based on 24 hours recall, each month cumulative % of breast milk attempt is within 75% of the acceptable limit	If based on 24 hours recall, each month cumulative % of breast milk attempt calculated is <75% ,it falls under the non per protocol limit

10. STATISTICAL ANALYSIS

General approach to data analysis

1. For the main study outcomes length velocity of the infant will be analyzed by predefined hypothesis in multiple arms. However conclusions on this part of the study will be based on formal hypothesis testing.
2. For secondary objectives (weight velocity, length-for-age, weight-for-length, and weight-for-age Z scores) will be analyzed by predefined hypothesis in multiple arms. However conclusions on this part of the study will be based on formal hypothesis testing.

Hypotheses to be tested

Primary

a) To assess the efficacy of a fortified, balanced energy-protein supplementation intake using a ready-to-use product to lactating women of Mid-upper-arm-circumference (MUAC) of less than 23.0 cm for initial 6 month of exclusive breastfeeding with or without single prophylaxis dose of Azithromycin to infants at day 42 of age, in improving length velocity as the primary outcome at 6 months of age as compared to the standard of care..

Hypothesis to be tested:

H_a = Mean difference in length velocity of >0.12cm/month to look at comparisons between multiple arms

H_o = Mean difference in length velocity of ≤ 0.12cm/month to look at comparisons between multiple arms

Secondary objective

a) To assess the efficacy of a fortified, balanced energy-protein supplementation intake using a ready-to-use product to lactating women of Mid-upper-arm-circumference (MUAC) of less than 23.0 cm for initial 6 month of exclusive breastfeeding with or without single prophylaxis dose of Azithromycin to infants at day 42 of age, on weight velocity, length-for-age, weight-for-length, and weight-for-age Z scores as secondary outcome at 6 months of age as compared to the standard of care.

Hypothesis to be tested:

i. **Growth velocity at 6 months of infant's age**

H_a = Mean difference in growth velocity of >0.4 gram/kg/day to look at comparisons between multiple arms

H_o = Mean difference in growth velocity of ≤ 0.4 gram/kg/day to look at comparisons between multiple arms

ii. Length-for-Age Z-score (LAZ) at 6 months of infant's age

H_a = Mean difference in LAZ >0.5 to look at comparisons between multiple arms

H_o = Mean difference in LAZ ≤0.5 to look at comparisons between multiple arms

iii. Weight-for-Length Z-score (WLZ) at 6 months of infant's age

H_a = Mean difference in WLZ >0.5 to look at comparisons between multiple arms

H_o = Mean difference in WAZ ≤0.5 to look at comparisons between multiple arms

iv. Weight-for-Age Z-score of (WAZ) at 6 months of infant's age

H_a = Mean difference in WAZ >0.5 to look at comparisons between multiple arms

H_o = Mean difference in WAZ ≤0.5 to look at comparisons between multiple arms

Data cleaning and procedures

Data cleaning process will be done by these procedures:

1. Data analyst will clean the required data for the main analyses. At this point, all investigators will be blinded to the intervention each participant has been receiving.
2. The study statisticians will review the data and complete preliminary analyses for group comparisons (without knowing the actual interventions).

Definition of key variables

i. Length velocity

Length velocity to be calculated from baseline length measurement of the infants taken within 0-6 days of life and subsequent measurement in 'cm' taken at each 4 weeks interval, until the age of 6 month, calculated as cm/month.

ii. Growth velocity

Growth velocity to be calculated from baseline weight measurement of the infants taken within 0-6 days of life and subsequent measurement in 'gram' taken at each 4 weeks interval, until the age of 6 month, calculated as gm/kg/month.

iii. Length-for-Age Z-score (LAZ) at 6 months of infant's age

Length-for-age will be calculated from age, sex, and length information from the baseline measurements within 0-6 days of birth and infant anthropometry on monthly basis until 6

months of age by using the STATA WHO packages using the WHO 2006 multi-centre growth standard. The values will be expressed as Z-score units, with two decimals.

iv. Weight-for-Length Z-score (WLZ) at 6 months of infant's age

Weight-for-length will be calculated from sex, weight and length information from the baseline measurements within 0-6 days of birth and infant anthropometry on monthly basis until 6 months of age by using the STATA WHO packages using the WHO 2006 multi-centre growth standard. The values will be expressed as Z-score units, with two decimals.

v. Weight-for-Age Z-score of (WAZ) at 6 months of infant's age

Weight-for-Age will be calculated from weight, sex and gender information from the baseline measurements within 0-6 days of birth and infant anthropometry on monthly basis until 6 months of age by using the STATA WHO packages using the WHO 2006 multi-centre growth standard. The values will be expressed as Z-score units, with two decimals.

Analysis approach

Statistical analysis will be performed by Stata, version 15. The baseline characteristics will be assessed by arm. The primary analysis will be an intention-to-treat (ITT) analysis. We will compare the mean length velocity (cm/ month) (primary outcome), weight velocity (gm/kg/day), LAZ, WLZ, and WAZ between the two intervention arms and the control arm using ANOVA, with the model adjusted for the birth weight and age of the infants at enrollment. If an outcome is missing for the intention-to-treat infants, the means from that group will be imputed.

Safety outcomes

i. Maternal serious adverse events

The occurrence of maternal SAEs will be expressed as the proportion of women with at least one SAE during the follow-up period (from enrolment to 179th day). The proportion will be calculated by dividing the number of women with at least one recorded SAE by the total number of enrolled participants. Results will be shown both as proportions of participants with any SAE as well as tabulated by the SAE category (death, hospitalization, other). If any participant has experienced more than one type of SAE, the participant will be recorded in each category.

ii. Infant serious adverse events

The occurrence of infant SAEs will be expressed as the proportion of babies with at least one SAE during the follow-up period (from enrolment to 179th day). The proportion will be

calculated by dividing the number of babies with at least one recorded SAE by the total number of recorded newborns. Results will be shown both as proportions of participants with any SAE as well as tabulated by the SAE category (death, hospitalization, other). If any participant has experienced more than one type of SAE, the participant will be recorded in each category.

Interim analysis

Data Safety and Monitoring Board of the trial will request an independent statistician to run interim analysis when 50% of the enrollment will complete the 6 months follow-ups in the trial. The analysis will be coded and the findings will on difference in primary, secondary outcomes and Serious Adverse Events will be shared to DSMB in blinded coding. The DSMB will review results from the interim data analysis and will determine whether stopping boundaries have been crossed. Additional interim analyses may be completed and reviewed by the DSMB, per the advisement of the DSMB chair. The DSMB may, at its own discretion, ask the interim analysis to be done earlier or additional interim analyses to be completed on an ad hoc basis.

For any interim analysis, the data management team at the VPT will prepare a blinded, cleaned analysis database. The interim analysis will be conducted on a blinded basis, as follows: Project data team at VPT will prepare and verify interim analysis programs using randomly generated dummy treatment assignments that have no relationship to the true intervention assignments. An independent statistician will subsequently incorporate blinded treatment codes group 1, 2 and 3 for safety end points status. The data team will separately provide treatment codes to the chair of the DSMB in a sealed file. At a minimum, the results to be presented will include a summary of screening and enrollment status, descriptive statistics of baseline variables, summaries of protocol violations, serious adverse events, primary, and secondary growth outcome. Site-specific and overall results will be pooled over treatment groups. DSMB may request other analyses as it deems fit. The DSMB will decide whether or not they wish to open the treatment code envelopes to un-blind themselves.

i. Stopping guidelines / Stopping rules

Based on the trial safety monitoring and interim analysis, the following may be the key decision/s of DSMB pertaining to the trial:

a) No action needed, trial continues as planned.

b) Early stopping due, for example, to clear benefit or harm of an intervention/s, uselessness, or external evidence.

c) Extending recruitment (based on actual control arm response rates being different to predicted rather than on emerging differences) or extending follow-up.
d) Stopping an arm of the multi-arm trial.
e) Sanctioning and/or proposing protocol changes

While stopping rules will be utilized to help the DSMB to assess the justification of trial continuation, the DSMB will take into account the balance of risks and benefits as well as consistency with external evidence. Thus, the DSMB recommendation to continue or discontinue will not be based on any single issue or value in any of the analyses, but a comprehensive analysis taking into account multiple issues around the trial.

The DSMB may recommend termination or modification of the study if:

i. In an interim analysis, there is strong evidence of a benefit (increases length velocity and/or secondary outcome) from the study intervention (O'Brien Fleming rule, $p<0.005$).
ii. In an interim analysis, there is strong evidence of harm (incidence of SAEs) from the study intervention. For harm assessment, no fixed statistical rules will be applied, but the DSMB will holistically consider point estimates and confidence intervals for SAE incidence differences, p-values from appropriate statistical tests and other relevant factors, when determining its recommendation about study continuation or discontinuation.

11. SAMPLE SIZE CALCULATIONS & POWER ANALYSIS

The sample size is calculated and powered to look at multiple comparisons, between arms A and B, A and C plus B and C with a hypothesized higher increase with addition of Azithromycin to infant with Afzaaish in LW. Also, adjustment for multiple comparison is needed. Thus, calculations for the sample size is estimated and are per arm sample size is provided below based on the primary outcome of length velocity in LW trial with 1-sided testing, alphas to account for multiple comparison (the lower alpha), and drop out of 14% is assumed in the trial.

Assumptions for Sample Size Calculations

Variable	Difference	Std Dev	Alpha	Drop out portion	n/arm	n/arm adj dropout	Actual Power
Length Velocity	0.12	0.5	0.025	0.14	274	319	0.801

Based on assumptions, a sample size of 319 children per arm is calculated, assuming 14% drop out rate (i.e. 10% margin for loss to follow up, and 4% infants deaths).

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