Statistical Analysis Plan for the effect of the intervention (early maternal and child supplementation) on hemoglobin of children at 4-6 y

Background of the Study

This study is a follow-up of children from a randomized controlled trial which was conducted in the Eastern Region of Ghana. The main study involved women who were at ≤20 weeks gestation and at least 18 years old. They were randomized to receive one of 3 treatments:

a. Daily iron and folic acid during pregnancy and a calcium tablet (placebo) during the first 6 months postpartum and no infant supplementation
b. Daily multiple micronutrient tablet (1-2 RDA of 18 vitamins and minerals) during pregnancy and first 6 months postpartum and no infant supplementation
c. Daily 20 g Lipid-based Nutrient Supplement (LNS) during pregnancy and first 6 months postpartum followed by infant LNS supplementation from 6-18 months postpartum. The maternal LNS had similar micronutrient content as the multiple micronutrient supplement plus calcium, magnesium, phosphorus, potassium and essential fatty acids, while the infant LNS has 22 micronutrients based on infant Reference Nutrient Intakes (RNIs).

All arms of the study were followed until infants were 18 months.

Children from the iLiNS Ghana trial were enrolled in this study when they were between 4 and 6 years. Data collected included socio-economic information to update the baseline information collected during the main trial, anthropometric data and hemoglobin by Hemocue.

The primary outcome for the follow-up study, when children were between 4 and 6 years, was height and height-for-age z-score (HAZ). In this analysis, we look at the effect of the intervention on hemoglobin of the children at 4-6 y. We use socioeconomic data, anthropometric data and hemoglobin measurements as part of this analysis.

Study Objective

The main objective of this analysis is to determine the effect of maternal (pregnancy and 6 months postpartum) and infant (6-18 months of age) LNS supplementation compared with two maternal nutritional supplements (iron and folic acid and a multiple micronutrient tablet) on hemoglobin status of the children from the iLiNS-DYAD Ghana trial at 4-6 y. In this analysis, we will also examine the background characteristics which may modify the intervention effects observed.

Hypotheses

a. Children in the LNS group will have higher hemoglobin status compared to children in the combined iron and folic acid and multiple micronutrient tablet group at 4-6 y

Outcomes and Definitions

The primary outcomes of this study are:

a. Hemoglobin status at 4-6 years

Secondary outcomes assessed at 4-6 years include:

- Anemia defined by the cutoff (<11 g/dl)
- Anemia defined by a lower cutoff (<10 g/dl)

Analysis Principles

Analysis will be based on the intention-to-treat principle. All tests will be two-sided, at a 5% level of significance.

Sample Size

The sample size was calculated based on detecting an effect size of 0.25 in mean height, resulting in a minimum sample size of 198 per group to detect the difference with 80% power. The same sample size calculation was used for other outcomes including hemoglobin.

Data Cleaning

Electronic data collection was employed in this study. The Open Data Kit (ODK) software was used to develop our data collection forms and data were collected using tablets. Limits for plausible values were built into the forms to prevent data collectors from entering implausible values. The data collectors were also provided a diary in which they entered the data for participants whose values were outside the limits. At the end of each week, the data manager ran data cleaning codes written by the statistician and any queries were addressed by the supervisor.

Blinding

The analyst for these analyses will remain blinded to group assignments until all decisions regarding outliers have been made and the initial run comparing treatment groups has been completed.

Statistical Analysis

Data will be analyzed using the SAS software version 9.4

Age of the child for analysis will be calculated based on the child's date of birth and date of data collection. Age of the child will be controlled for in all models.

All data obtained will be examined using univariate analysis (graphical plotting) to look at outliers. We will check outliers by visually inspecting Box plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers which are clearly impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers which are plausible or possible will be kept. In analysis of secondary outcomes, variables with outliers will be transformed, and in an extreme situation, a sensitivity analysis will be done to determine if such outliers have undue influence on the results.

Continuous outcomes will be assessed for conformance to the normal distribution and will be transformed appropriately. If no suitable transformation can be found to optimize normality and homogeneity of variances, analysis will be done on ranked data.

The effect of the intervention analysis will begin with testing the null hypothesis of no difference between the two groups using ANCOVA and controlling for pre-specified covariates including

baseline values. Categorical variables will be analyzed using logistic regression also controlling for covariates. Only covariates significantly associated with an outcome at 10% level of significance in a bivariate analysis will be include in the final adjusted analysis which may result in different sets of covariates for each outcome. See below for the list of covariates.

The effects of potential effect modifiers will be assessed with an interaction term in the ANCOVA or logistic regression model. Significant interactions ($p < 0.05$) will be further examined with stratified analyses, estimation of separate regression lines, or estimation of adjusted means at key points of the covariate, in order to understand the nature of the effect modification.

A sensitivity analysis will be performed comparing the 3 groups to rule out differences between the IFA and MMN groups.

Covariates: Sex of child
Age of child at follow up
Gestational age at enrollment
Primiparity
Maternal education at enrollment
Maternal BMI at enrollment
Maternal anemia
Maternal zinc protoporphyrin (ZPP) at enrollment
Maternal height
Asset score at enrollment

Effect modifiers: Sex of child
Primiparity
Maternal education at enrollment
Maternal BMI at enrollment
Maternal anemia
Asset score at enrollment

Presentation of Results

Table 1: Background characteristics of women and children from the main trial

Variable	IFA+MMN $\bar{x} \pm SD$ [n]	LNS $\bar{x} \pm SD$ [n]	p-value
Maternal Characteristics			
Age (y)			
Gestational age at enrolment (wk)			
Years of formal education			
Married or Cohabiting (% [n])			
Asset score			
Primiparous women (% [n])			
Weight (kg)			
Height (cm)			
BMI (kg/m^2)			
Underweight (BMI <18.5) (% [n])			
Mid upper arm circumference (cm)			
Triceps skinfold (mm)			
Haemoglobin (g/L)			
Anaemia (% [n])			
Iron deficiency anaemia in pregnancy (% [n])			
Child Characteristics			
Current age of child (y)			
Sex of child (%)			
Height at 4-6 y			
Weight at 4-6 y			
BMI at 4-6 y			

Table 2: Comparison of continuous hemoglobin measurements

	IFA+MMN $\bar{x} \pm SD$ [n]	LNS $\bar{x} \pm SD$ [n]	p-value	Difference in mean (95% CI)
Hemoglobin (g/dl)				

Table 3: Comparison of categorical outcomes

	IFA+MMN [n]	LNS [n]	p-value
Anemia (Hb <11 g/dl)			
Prevalence (%)			
OR (95% CI)			unadjusted adjusted
Anemia (Hb <10 g/dl)			
Prevalence (%)			
OR (95% CI)			unadjusted adjusted