Examining the Relationships Among Maternal Exposure to Aflatoxins, Birth Outcomes and Stunting in Nepalese Infants: Protocol for the AflaCohort Birth Cohort Study


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Background

Aflatoxins, carcinogenic food-borne toxins, have been associated with stunting in children in sub-Saharan Africa. However, research has not examined their potential effects on linear growth, controlling for other potential explanatory factors.

Study Objectives and Hypotheses

The AflaCohort Study runs from 2015 to 2018. A total of 1,675 pregnant women ages 16-49 were recruited from 17 Village Development Committees in the Banka district. This longitudinal birth cohort examines the associations among socio-demographic, crop management practices, aflatoxin prevalence, birth outcomes and stunting in children in Nepal. Additionally, the study will validate dried blood spots as a less invasive, low cost collection method compared to venous blood samples.

The specific aims of this study are:
1. To examine the relationship of maternal mycotoxin exposure in pregnancy and birth outcomes, including infant birth weight.
2. To examine the relationship of exposure to mycotoxin of infants through breast milk and their linear growth.
3. To examine the relationship of exposure to mycotoxin through complementary feeding and linear growth.
4. To examine the relative contributions of maternal and infant mycotoxin exposures in impairing linear growth, controlling for other potential explanatory factors.

Hypotheses

1. There is an incremental effect of in utero, lactation and complementary feeding mycotoxin exposures on rate of length gain and stunting outcomes for age z-scores in infants at 1 year of age.
2. Maternal exposure to mycotoxins will be significant predictor of birth weight in infants, thereby being a significant contributor to the burden of stunting at 1 year of age.
3. Exposure to mycotoxins through breast milk before 6 months of age along with continued exposure through both breast milk and complementary foods (after 6 months of age) is a significant contributor to the burden of stunting at 1 year of age.
4. Improper farm management, food processing and storage practices are significantly related to higher levels of serum mycotoxins in the blood of mothers and their infants.
5. Knowledge of the problem of food-borne contaminants is associated with improved food processing and storage practices.

Methodology

Participants are visited a total of 7 times: 1) pre-natal 2) birth 3) 3 months 4) 6 months 5) 9 months 6) 12 months of age and 7) one year post baseline.

HOUSEHOLD SURVEY: Electronic questionnaires are administered by trained enumerators to collect data on risk factors for aflatoxin exposure and stunting, such as socio-economic status, maternal and child diet, education, sanitation and hygiene, agricultural grain drying and storage practices.

ANTHROPOMETRY: The study collects maternal and infant anthropometry data at all biological time points. Within 72 hours of birth, the study team collects data on infant length and weight. Length, weight, MUAC, head circumference and knee heel length data are collected at 3,6,9 and 12 months of age.

AFLATOXIN EXPOSURE ASSESSMENT:

- Maternal venous blood sample (3-5 ml) collected once during pregnancy.
- Infant venous blood samples (1-3 ml) collected three times (3.6, and 12 months).
- Dried Blood Spots (DBS) collected once during pregnancy and infants at 3.6, and 12 months. The study uses Whatman filter paper to randomly collect DBS from a subset (n=1200) of mothers and infants at different times to compare with venous blood samples.
- HPLC: The assessment of aflatoxin B1 in serum and the validation of DBS are conducted at the University of Georgia using a high-performance liquid chromatography (HPLC)-fluorescence detection method.
- Aflatoxin M1:
- Breast milk sample (25-50 ml) collected once when infant is 3 months of age.
- ELISA: Aflatoxin M1 concentrations in breast milk will be measured locally using enzyme-linked immune-sorbent assay (ELISA).

Methodology Continued

SAMPLE PROCESSING AND STORAGE:
- Samples are stored in cool boxes and transported on the same day to the laboratory in Kohalpur, Banke. There the blood was allowed to clot at room temperature and subsequently centrifuged at less than 5000 RPM for 10 minutes and frozen at -20°C or lower.
- Samples are shipped on a weekly basis to a -80°C freezer stored at the Patan Academy for Health Sciences (PAHS) in Kathmandu.
- 400-microliter aliquots destined for aflatoxin analysis are air-shipped, on dry ice, to the University of Georgia for aflatoxin-albumin (AF-alb) adducts analysis.

Early Findings

Conclusions and Future Work

This study will be the first to document the cumulative effects of aflatoxin exposure in pregnancy and its association with child growth.

Findings from the study will provide information on alternative, less invasive aflatoxin testing methods as well as identify certain factors that warrant interventions to reduce aflatoxin-related stunting in Nepal.

The prospective longitudinal birth cohort design offers an opportunity to investigate a complex system of exposures during the infant’s first year of life.

In July 2017, all samples for time point 3 (infant 3 months of age) will be shipped to UGA for AF-alb testing.

Study results for all time points are anticipated by the end of 2018.