ENVIRONMENTAL ENTERIC DYSFUNCTION SUB-STUDY SUMMARY

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BACKGROUND

Recent studies have shown that EED contributes to chronic malnutrition (stunting) and chronic inflammation of the intestine, which could also affect growth in children. The alterations to the small intestine associated with EED (reduced barrier integrity, absorptive capacity, and inflammation) pose a serious problem because they lead to poor digestion and absorption of nutrients by the small intestine. [1]

The most commonly used method to detect EED is the dual sugar test: a mixture of lactulose and mannitol is given and the ratio of these two sugars in the urine is assessed to determine functional capacity of the small intestine. However, the dual sugar test has a high participant burden due to the 4 hours required to remain on site to provide the post-dose urine specimen. Thus, newer markers of EED have been explored; one promising marker is fecal mRNA. A recent study showed that seven markers may be used to determine severe EED.

These mRNA transcripts and their functions are CD53 (cell adhesion), CDX1 (intestinal differentiation), HLA-DRA (adaptive immune response), MUC12 (epithelial barrier function), REG1A (regeneration of epithelial cells), S100A8 (innate immune response), and TNF (innate immune response).[2]

STUDY DESIGN

Eligible subjects will be children with MAM ages 6-59 months enrolled in the four foods study. For each food, 200 children will be enrolled during specific study time periods: a total sample size of n=800. Informed consent will be sought from the caretakers of all eligible children by a research assistant familiar with the study and fluent in the caretaker's native language. Duration of the study and participation requirements will be explained. Informed consent will be given verbally and in writing.

AIM 2

[1] Crane, R. J., Jones, K. D. & Berkley, J. A. Environmental enteric dysfunction: an overview. *Food and Nutrition Bulletin* 36, S76-87 (2015).
[2] Ordiz, M. I. *et al.* Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal mRNAs. *Journal of Pediatric Gastroenterology and Nutrition* **63**, 453-459, doi:10.1097/mpg.0000000000001315 (2016).

To examine whether EED modifies the effect of four supplementary foods on 6-59 month-old children with MAM.

HYPOTHESIS 2A

The presence of EED at baseline differently modifies the effect of the 4 study foods on recovery of 6-59 month-old children from MAM within 12 weeks.

HYPOTHESIS 2B

The 4 foods have differential effects on change in prevalence and severity of EED of 6-59 month-old children with MAM after four weeks of treatment.

MEASUREMENTS

The outcome measure for hypothesis 2a is the primary study outcome, recovery from MAM within 12 weeks. EED will be measured through fecal host mRNA: CD53, HLA-DRA, MUC12, CDX1, S100A8, REG1A, and TNF at baseline and after four weeks of treatment using digital droplet polymerase chain reaction (ddPCR). To validate the fecal mRNA measures, EED will also be assessed by the lactulose mannitol ratio at baseline in a sub-set of children using high-performance liquid chromatography (HPLC).

TECHNIQUE

To assess EED we will use two methods: dual sugar test and fecal host mRNA. For the dual sugar test, we will provide children with a sugar solution of lactulose and mannitol and collect all urine excreted over the next 4 hours. These will then be aliquoted in 2mL cryovials, flash frozen in liquid nitrogen, and placed in a -80oC freezer until shipped to a laboratory for analysis. For the fecal host mRNA children will be given a disposable diaper and fresh stool samples will be collected and placed in 2mL cryovials. These will also be flash frozen in liquid nitrogen and placed in a -80oC freezer until shipped for analysis.

IMPLICATIONS

The results from this study will contribute to understanding whether EED modifies the effectiveness of the four products in recovery from MAM and whether the foods promote healing of EED differentially. Furthermore, the study provides an opportunity to develop a scale for EED using novel fecal host mRNA markers.