The gut microbiome and metabolome profiles in HIV- exposed uninfected infants in Kenya

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Background

The huge success of Mother-to-Child HIV prevention programs has seen a growing population of HIV-exposed uninfected (HEU) infants. **In 2018, there were an estimated 14·8 million (11·1–18·3) children who were HEU, 13·2 million (9·8–16·3; 90%) of whom resided in sub-Saharan Africa.** Although these infants are born HIV-free, they have altered immunity during infancy have a higher prevalence of stunting, poor growth outcomes and are more susceptible to infections than HIV unexposed uninfected (HUU) infants.

Fig 1: Estimates of the contribution of individual countries to the global population of children who were HEU in 2018

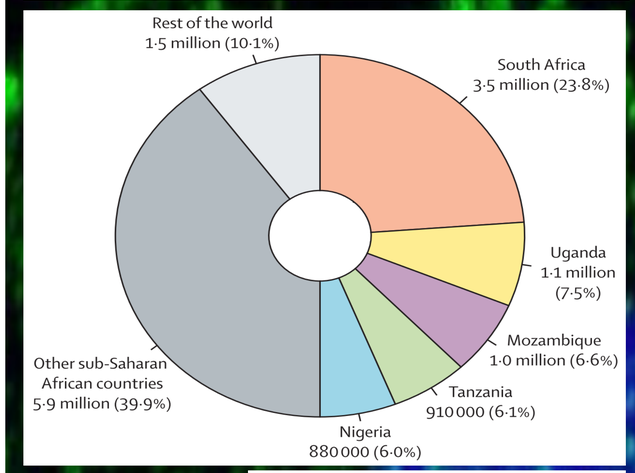

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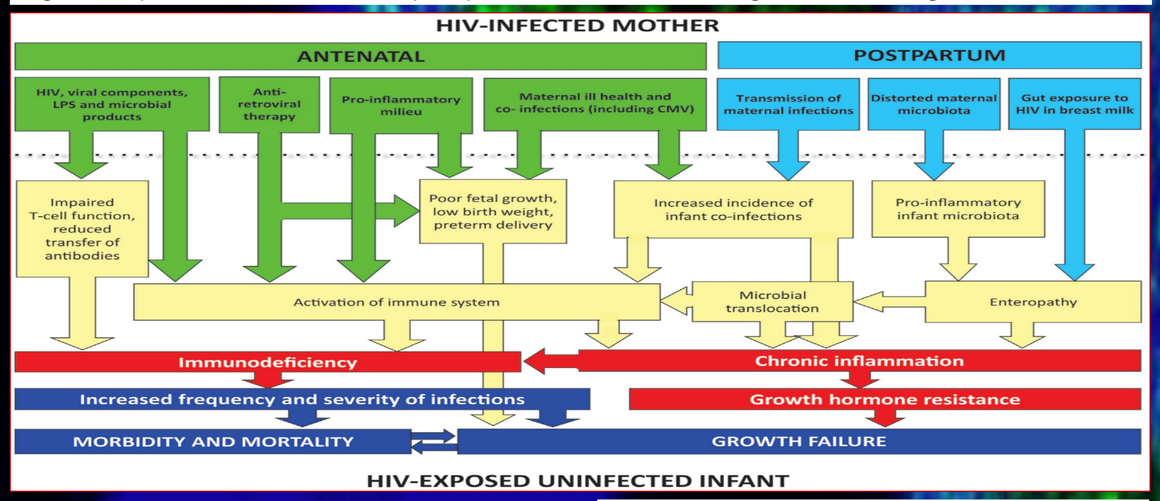
Fig 2: Proposed anternatal and postpartum contributors of growth faltering in HEU infants



Evans et al., 2016, Frontiers in Immunology

Therefore, there is a pressing need to understand and address the Biochemical mechanisms underlying compromised outcomes in these infants using a multi-omics approach. The central **hypothesis** is that HIV exposure *in utero* alters the microbiome in HIV-infected mothers which is passed to the infant consequently altering infants' microbiome, metabolome during early life causing growth faltering. Using 24 HEUs and 24 HUUs, three specific aims are proposed to:

1) **Describe the composition of gut microbiome between HEU and HUUs**
2) **Describe the infant gut metabolome profile between HEU and HUUs**
3) **Define the growth trajectory using microbiota-for-age Z-score (MAZ) between HEUs and HUUs at 3, 6 and 12 months of life**.

Methods

Study Design: **Prospective Cohort**
Study site: KN**H pediatric clinics and KAVI-ICR Laboratory**
Study population: **24 HEUs and 24 HUUs(controls)**
Inclusion criteria: **<1 month old infants**
Exclusion criteria: **Presence of severe diarrhea in the past 30 days**

Aim 1: **Gut microbiome of HEUs vs HUUs**

Stool collection
↓
Microbiota DNA extraction — Nycodenz®
↓
16S rRNA sequencing — V3-V4 amplicon Novaseq 6000
↓
Diversity Analysis — (α and β diversity)
↓
Function

Aim 2: **Gut metabolome of HEUs vs HUUs**

Stool collection
↓
Extraction — 70% MeOH
↓
Mass Spectrometry — UPHPLC, LC-MS/MS
↓
Processing — XCMS, MZMINE3
↓
Statistics — MetaboAnalyst: PLS-DA, ANOVA, and Heatmaps
↓
Pathway Identification — Metlindatabase search, KEGG pathway analysis
↓
Role in Disease — Hypothesis generation

Aim 3: compute **Microbiota-for-age Z-score (MAZ)** for HEUs vs HUUs

$$\frac{(\text{microbiota age} - \text{median microbiota age of healthy children of same chronologic age})}{(\text{s.d. of microbiota age of healthy children of the same chronologic age})}$$

Statistical Analysis: A **Linear mixed effects** model will examine the relationship between gut microbiome (aim 1) and metabolome (aim 2) on a longitudinal response - infant's growth (aim 3) at 3, 6, and 12 months.

Integration of Microbiome and Metabolome datasets: Multi-Omics Factor Analysis (MOFA)

Anticipated Results

An integrated view of the role of the the microbiome and metabolome in growth faltering and the the proportion of variance explained by each omics dataset.

A longitudinal study design from the same set of subjects at three different time points, will allow for a more accurate representation of higher-order interactions and associated variability across time as well as distinguish differences between subjects from changes within subjects.

While association does not imply causation, knowing how HIV-exposure associated microbiome and metabolome correlates with growth and development in infants in LMIC settings is worthwhile. This information is critical for identifying potential mechanisms underlying the failure to thrive and disease burden in HEU infants and reveal potential areas for targeted intervention to improve health outcomes.

Acknowledgements

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