The Gut Microbiome of Undernourished Indian Children

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India
Quiz Time

http://quiz.wfp.org/
Question 1:

Which region has the greatest number of hungry people?

- Africa
- Asia and the Pacific
- Latin America and the Caribbean
- Middle East

The answer is Asia and the Pacific, which is home to 578 million of the world’s hungry, compared to 239 million in Sub-Saharan Africa and 53 million in Latin America and the Caribbean.
Question 2:

Which of the following is the No. 1 health risk in the world?

- AIDS
- Hunger
- Malaria
- Tuberculosis

Hunger kills more people every year than AIDS, malaria, and tuberculosis combined.
Question 3: How much does it take to feed a schoolchild for a day?

- $1.00
- $0.75
- $0.50
- $0.25

It takes only $0.25 for the World Food Programme to give a hungry schoolchild a cup of nutritious food. $50 will feed a child at school for an entire year.
The number of malnourished people in the world is equal to:

- The population of the United States
- The population of the European Union
- The population of Canada
- The population of all of the above, combined

The answer is the population of the US, Canada, and the EU combined. The number of malnourished people worldwide is just under 1 billion people – nearly a seventh of the global population.
Question 5:

True or False: There is enough food in the world for everyone.

- True
- False

The statement is true! There is enough food today for everyone to have the nourishment needed for a healthy and productive life - the problem is access.
Outline of the presentation

• Undernutrition: causes, associated problems, assessment and global and national prevalence

• Is the gut microbiota altered in undernourished children?

• Gut microbiome of Indian children of varying nutritional status

• Take home messages
Undernutrition: causes, associated problems, assessment and global and national prevalence
What is undernutrition??

**Undernutrition** is defined as the outcome of insufficient food intake and repeated infectious diseases. It includes being underweight for one's age, too short for one's age (stunted), dangerously thin for one's height (wasted) and deficient in vitamins and minerals (micronutrient malnutrition).

Worsens and perpetuates each other as a result of impaired innate and adaptive immune responses.

**Vicious cycle of undernutrition**

- Malabsorption
- Inadequate dietary intake
- Impaired immune responses
- Enteric infections
- Altered microbiota
- Dysregulated gut permeability

Relman, D.A. 2013 Science; 339:530-532
The vicious cycle of diarrhoea and undernutrition in susceptible children
Assessment of undernutrition

- **Anthropometric indicators**
  - ✓ (most common and easiest to use)
- **Biochemical indicators**
  - ✓ (blood monitoring)
- **Clinical indicators**
  - ✓ (physical manifestations of nutrient deficiencies)
## Assessment of Anthropometric measures

<table>
<thead>
<tr>
<th>Anthropometric Indicators</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stunting</strong> (too short for one’s age)</td>
<td>Height-for-age Z-score (HAZ)</td>
</tr>
<tr>
<td><strong>Underweight</strong> (Low weight for one’s age)</td>
<td>Weight-for-age Z-score (WAZ)</td>
</tr>
<tr>
<td><strong>Wasting</strong> (dangerously thin for one’s height)</td>
<td>Weight-for-height Z-score (WHZ)</td>
</tr>
</tbody>
</table>
Percentage of children under age 5 who are moderately or severely stunted

165 million children <5 yrs were stunted
101 million children <5 yrs were underweight
52 million children <5 yrs were wasted

UNICEF Global Nutrition Database, 2012

Note: Data are from 2007 to 2011, except for India.
Percentage of children under age 5 who are moderately or severely wasted

<table>
<thead>
<tr>
<th>Ranked by burden (2011)</th>
<th>Country</th>
<th>Year</th>
<th>Wasting (% moderate or severe)</th>
<th>Wasting (% severe)</th>
<th>Number of wasted children, 2011 (moderate or severe, thousands)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>2005-2006</td>
<td>20</td>
<td>6</td>
<td>25,461</td>
</tr>
<tr>
<td>2</td>
<td>Nigeria</td>
<td>2008</td>
<td>14</td>
<td>7</td>
<td>3,783</td>
</tr>
<tr>
<td>3</td>
<td>Pakistan</td>
<td>2011</td>
<td>15</td>
<td>6</td>
<td>3,339</td>
</tr>
<tr>
<td>4</td>
<td>Indonesia</td>
<td>2010</td>
<td>13</td>
<td>6</td>
<td>2,820</td>
</tr>
<tr>
<td>5</td>
<td>Bangladesh</td>
<td>2011</td>
<td>16</td>
<td>4</td>
<td>2,251</td>
</tr>
<tr>
<td>6</td>
<td>China</td>
<td>2010</td>
<td>3</td>
<td>–</td>
<td>1,891</td>
</tr>
<tr>
<td>7</td>
<td>Ethiopia</td>
<td>2011</td>
<td>10</td>
<td>3</td>
<td>1,156</td>
</tr>
<tr>
<td>8</td>
<td>Democratic Republic of the Congo</td>
<td>2010</td>
<td>9</td>
<td>3</td>
<td>1,024</td>
</tr>
<tr>
<td>9</td>
<td>Sudan</td>
<td>2010</td>
<td>16</td>
<td>5</td>
<td>817</td>
</tr>
<tr>
<td>10</td>
<td>Philippines</td>
<td>2008*</td>
<td>7</td>
<td>–</td>
<td>769</td>
</tr>
</tbody>
</table>

*Data differ from the standard definition or refer to only part of a country.

Is the gut microbiota altered in undernourished children?
Intestinal microbiota of a malnourished child

Gupta et al. Gut Pathogens 2011, 3:7
http://www.gutpathogens.com/content/3/1/7

Metagenome of the gut of a malnourished child

Sourav Sen Gupta¹, Monzoorul Haque Mohammed², Tarini Shankar Ghosh², Suman Kanungo¹, Gopinath Balakrish Nair¹ and Sharmila S Mande²*
Taxa/lineages overabundant in malnourished and healthy child gut genomes
Overall differences between microbial communities residing in the gut of a malnourished and a healthy child.
Key Message

The intestinal microbiota of the malnourished child when compared to the healthy child is interpreted as aberrant gut microbiota extending the understanding of the basis of malnutrition beyond nutrition deprivation.
Gut Microbiomes of Malawian Twin Pairs Discordant for Kwashiorkor

Highlights of the Malawian Study

(i) the gut microbiome provides essential functions needed for healthy postnatal growth and development

(ii) disturbances in microbiome assembly and function (e.g., those prompted by enteropathogen infection), affect the risk for kwashiorkor; and

(iii) in a self-reinforcing pathogenic cascade, malnutrition affects gut Microbiome functions involved in determining nutritional status, thus further worsening health status.
Persistent gut microbiota immaturity in malnourished Bangladeshi children

Sathish Subramanian¹, Sayeeda Huq², Tanya Yatsunenko¹, Rashidul Haque², Mustafa Mahfuz², Mohammed A. Alam², Amber Bezerra¹,³, Joseph DeStefano¹, Martin F. Meier¹, Brian D. Muegge¹, Michael J. Barratt¹, Laura G. VanArendonk¹, Qunyuan Zhang⁴, Michael A. Province⁴, William A. Petri Jr⁵, Tahmeed Ahmed² & Jeffrey I. Gordon¹

Immaturity in the gut microbial community

Undernourished children fall behind not only on growth, but also on maturation of their intestinal bacterial communities, according to a study comparing acutely malnourished and healthy Bangladeshi children. See Letter p.417
Key Findings

• SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following two widely used nutritional interventions.

• Immaturity is also evident in less severe forms of malnutrition and correlates with anthropometric measurements.

• Microbiota maturity indices provide a microbial measure of human postnatal development, a way of classifying malnourished states, and a parameter for judging therapeutic efficacy.

• More prolonged interventions with existing or new therapeutic foods

• Next-generation probiotics using gut-derived taxa may also be required in addition to food-based interventions
Gut microbiome of undernourished Indian children
Gut Microbiomes of Indian Children of Varying Nutritional Status

Tarini Shankar Ghosh\textsuperscript{1,9}, Sourav Sen Gupta\textsuperscript{2,9}, Tanudeep Bhattacharya\textsuperscript{1}, Deepak Yadav\textsuperscript{1}, Anamitra Barik\textsuperscript{3}, Abhijit Chowdhury\textsuperscript{3–4}, Bhabatosh Das\textsuperscript{2}, Sharmila S. Mande\textsuperscript{1*}, G. Balakrish Nair\textsuperscript{2*}

1 Bio Sciences R&D, TCS Innovation Labs, Pune, India, 2 Center for Human Microbial Ecology, Translational Health Science and Technology Institute, Gurgaon, India, 3 Society for Health and Demographic Surveillance, West Bengal, India, 4 Department of Hepatology, School of Digestive and Liver Diseases, Institute of Post Graduate Medical Education and Research, Kolkata, India

West Bengal

Birbhum district of West Bengal which is typical of rural and agricultural setting.
Birbhum Population Project is a health and demographic surveillance system – longitudinal observations
# Anthropometric measures and metadata of the subjects

<table>
<thead>
<tr>
<th>Anthropometric measures</th>
<th>Category</th>
<th>Values</th>
<th>Overall nutritional status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-score 1</td>
<td>Height for age</td>
<td>-0.63 to -4.16</td>
<td></td>
</tr>
<tr>
<td>Z-score 2</td>
<td>Weight for age</td>
<td>-1.07 to 4.32</td>
<td></td>
</tr>
<tr>
<td>Z-score 3</td>
<td>Weight for height</td>
<td>1.6 to -3.10</td>
<td></td>
</tr>
<tr>
<td>Cumulative Z-score</td>
<td></td>
<td>-11.58 to -2.18</td>
<td>apparently healthy &gt; -6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>borderline malnourished -9 &lt; to &lt; -6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>severely malnourished &lt; -9</td>
</tr>
</tbody>
</table>

Based on the cumulative nutritional index, the 20 gut metagenomes were divided into three groups as AH, BM and SM.
Taxonomic composition of the 20 gut microbiomes

Across 20 microbiomes 72% assigned at phylum level. 8 phyla present but only 4 dominant

- **Bacteriodetes**
- **Firmicutes**
- **Proteobacteria**
- **Actinobacteria**

**Spirochaetes**, **Fusobacteria**, **Synergistetes** and **Euryarchaeota**

36.2% assigned at Genus level*

4.2% (1.4% to 12.4%) assigned at species level
Variation of the rank normalized abundances of the 23 core genera across the 20 metagenomes
Gut Microbiome

Bacteroides dominated enterotypes

Prevotella dominated enterotypes

An enterotype is a classification of living organisms based on its bacteriological ecosystem in the human gut microbiome. Enterotypes are not dictated by age, gender, body weight, or national divisions.

Arumugam et al., 2011, Nature
Heat map showing the normalized rank abundances of 23 genera and 8 phyla across the 20 gut microbiomes.

Metagenomes are arranged in the bottom panel (from left to right) in decreasing order of their cumulative nutritional scores. Taxa are arranged vertically on the left panel as a hierarchical tree based on the similarities in their abundance patterns.
Box-plots showing the abundances of the taxonomic groups significantly differing across the three groups (AH, BL, SM) of gut microbiomes.
**Genera co-occurrence networks** obtained for gut microbiomes

Despite having contrasting trends in abundance, some of them showed strong positive associations amongst each other.

With decrease in NS the pathogenic genera come together in a single connected hub.

The increasing interdependence among the genera is even more pronounced for the SM group of metagenomes.
Comparison of the relative abundances of the different functional categories
Glycoside hydrolases encoded by the human genome

Carbohydrate-active enzyme composition of bacterial mini-microbiome

- Glycoside hydrolases
- Polysaccharide lyases
- Glycosyltransferases
- Carbohydrate esterases

GH families
- GH1, GH13, GH31, GH37
- GH1, GH9, GH18, GH31, GH35

Other GHs
- Lysosomal GHs
- GHs for processing of host N-glycans
- GHs involved in tissue development
- GHs for defence against pathogens
- GH-like proteins with regulatory functions

- Non-digestive
- Digestive
- Possibly digestive

El Kaoutari et al., 2013
Abundance patterns of CAZyme families across gut metagenomes with varying nutritional status

Gr 3 and Gr 6 CAZyme families are known degrading complex plant carbohydrates while Gr 7 are mostly peptidoglycan degraders.
Key Messages

• Link between the gut microbiome and the nutritional status of children in the Indian setting
• Impaired nutritional status is not only due to the abundances of likely pathogenic microbial groups, but also a result of depletion of several commensal genera
• Certain functional categories (COG groups) are positively/negatively correlated with nutritional status
• Functional groups positively correlated relate to nutrients utilization while negatively correlated functional groups may initiate the infection process
• Higher number of virulence genes in children with lower nutritional index
Key Messages

• CAZyme families that degrade peptidoglycans and complex plant carbohydrates associated with higher nutritional index

• Identification of distinct changes in general co-occurrence networks with progressive decrease in the nutritional status of the children is a key finding

• Modulate gut microbiome by disrupting certain key players in order to achieve conditions that could result in the regression of the disease phenotype

• More comprehensive and well designed functional studies are required for formulating a microbial basis of therapy for severe acute undernutrition
Study members

Sharmila S. Mande
Tarini S. Ghosh
Bhabatosh Das
Sourav Sen Gupta
Abhijit Chowdhury