The Gut Microbiome and Immune Function During Pregnancy in Cebu, Philippines

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BACKGROUND

The present study investigates the effect of pregnancy on the gut microbiome (GM) and immune function.

Immune Function Changes are Essential for Pregnancy[1,2]

Trimester	Inflammation	Cause
1	High	Embryo implantation
2	Low	Fetus Growth
3	High	(Preparation for) Childbirth

The GM May Play a Role in the Production of the Immune Response

- Variation in the GM is associated with immune diseases like multiple sclerosis and inflammatory bowel disease.[3]
- The GM modulates immune function through the production of **short chain fatty acids (SCFAs),** which are byproducts of the fermentation of dietary fiber by the GM.
- SCFAs activate different components of the immune system that secrete proteins involved in the pro- and anti- inflammatory response.
- These proteins, commonly referred to as **immune markers (IMs),** include **cytokines**, a class of signaling molecules, and **C-reactive protein (CRP)**, whose concentration rises in response to inflammation.[4]

AIMS & HYPOTHESES

AIM: Examine if and to what extent immune function and the GM change during pregnancy

H1: The GM differs in diversity and composition during pregnancy
H2: IMs differs between pregnant and non-pregnant women
H3: IM variation correlates with GM changes

METHODS

- **Samples:** Fecal samples and blood spots were collected from 49 pregnant and 50 non-pregnant women participating in the Cebu Longitudinal Health and Nutrition Survey (CLHNS) in 2018-2019. Survey data describing current health, environment, and socioeconomic status was also collected.
- **GM Analyses:** Microbial taxonomic composition of fecal samples was determined using 16s rRNA gene sequencing. QIIME2, a microbiome informatics platform, was used to quality filter the data and identify unique DNA sequences. Statistical analyses in R was used to evaluate microbial diversity and composition.
- **IM Analyses:** IMs of blood spots were originally supposed to be quantified using enzyme-linked immunosorbent assays (ELISAs). Due to Covid-19, these samples are unable to be shipped to the US until Summer 2021. In the meantime, IM data collected from the same women in 2005 was used to perform IM analyses. Although it is not optimal to use data collected at two different time periods, baseline values for IMs should be stable enough to make this comparison.
- **Correlation:** Linear models were used to assess the relationship between IMs and GM composition and diversity.

RESULTS

Pregnancy does not have a significant effect on the gut microbiome.

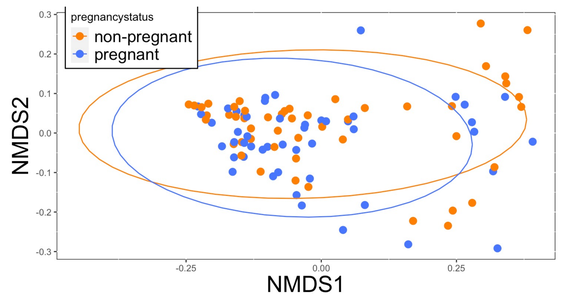

Figure 1: Non-metric Multi-dimensional Scaling (NMDS) plot using Weighted UniFrac indicates pregnancy does not explain the variation in this dataset (r^2 = 0.00252, p > 0.05), even when controlling for confounding factors such as socioeconomic status and hygiene.

Immune markers do not differ between pregnant and non-pregnant women.

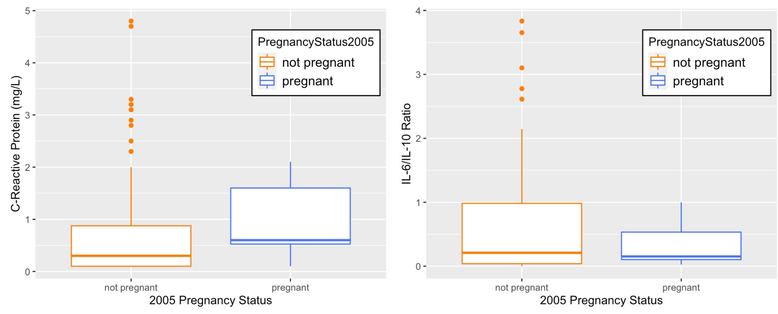

Figure 2: There is no significant difference in C-reactive protein levels (left) and the ratio of cytokines IL-6 to IL-10 (right) between pregnant and non-pregnant women in blood spots collected in 2005 (p > 0.05).

In non-pregnant women, there is no correlation between gut microbiome diversity and immune marker levels.

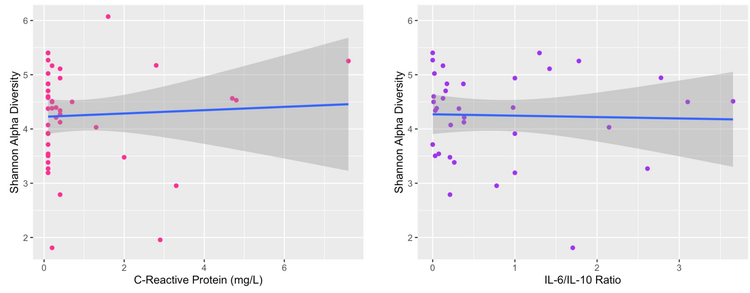

Figure 3: There is no correlation between Shannon Alpha Diversity, a measure of microbiome diversity, and 2005 C-reactive protein levels (left) or the ratio of cytokines IL-6 to IL-10 (right) in non-pregnant women (p > 0.05). It is important to note that GM diversity was calculated using fecal samples collected in 2018/2019, while IM levels were quantified using blood spots from 2005.

DISCUSSION

- Contrary to previous studies, pregnancy does not have a significant effect on the GM in this dataset.
  - Since most previous research on immune function and the GM during pregnancy focused on American and European populations, these results suggest that the relationship between these factors vary across populations.
- Immune markers do not differ between pregnant and non-pregnant women.
  - This result may be attributable to small sample size, since the 2005 IM dataset had significantly less pregnant women than the original 2018/2019 dataset.
- In non-pregnant women, there is no correlation between GM diversity and immune marker levels.
  - Since the GM data was collected from fecal samples in 2018/19, while the IM data was collected from blood spots in 2005, this result may be attributable to the large time gap.

FUTURE DIRECTIONS

- Control for additional confounding variables like diet and overall health that may be obscuring the effect of pregnancy on the GM.
- Redo the IM and correlation analyses with 2018/2019 IM data (when it arrives) to see if results change.
- Measure SCFAs in existing fecal samples to better understand whether these metabolites modulate the relationship between inflammation and the GM.

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