AMMI Canada Medical Student Research Proposal

Project title: *Impact on gut bacterial composition in Cambodian women receiving daily iron supplementation with either ferrous sulfate or ferrous bisglycinate: a 12-week randomized controlled trial*

Applicant: Emma Finlayson-Trick[1]

Supervisor: Drs. David Goldfarb[1,2] and Crystal Karakochuk[3]

[1] Faculty of Medicine, University of British Columbia, Vancouver, Canada

[2] British Columbia Children's Hospital Research Institute, Vancouver, Canada

[3] Department of Food, Nutrition and Health, University of British Columbia, Vancouver, Canada

Background

Iron is essential for life for many living organisms. As such, dysregulation of iron (in the form of iron overload or anemia) can result in deleterious effects throughout the body. For example, since the 1980's, studies have shown that gut bacterial composition changes when iron-deficient individuals receive iron supplementation (1–3). However, it is unclear whether iron supplementation preferentially increases enteric pathogens. *In vitro* work by Kortman et al. demonstrated that *Salmonella* has an increased ability to invade intestinal epithelial cells when moderate extracellular iron levels are available (4). This is in contrast, however, to work by Sanchez et al. that showed a reduced pathogenicity of *Citrobacter* in a mouse infection model supplemented with iron (5). Understanding how iron supplementation impacts enteric pathogens will be important as the global push to reduce anemia is intensified.

The World Health Organization (WHO) has a Global Nutrition Target for 2025 to reduce anemia in women of reproductive age by 50%. Beginning in 2016, the WHO began recommending oral iron supplements (ferrous sulfate 60 mg daily for 12 weeks) for all women where anemia prevalence was more than 40%, such as in Cambodia. The majority of Cambodian women, however, do not have anemia related to iron-deficiency, but to genetic blood disorders (6). Consequently, iron supplementation is estimated to only benefit approximately 10% of Cambodian women (6).

The bioavailability of iron used for supplementation greatly impacts the effectiveness of treatment. Ferrous sulfate, the common form of iron supplement recommended by the WHO, has a comparatively low bioavailability to ferrous bisglycinate, a newer form of supplement (7).

Furthermore, ferrous bisglycinate is associated with fewer gastrointestinal side-effects (7). Consequently, We hypothesize that women who receive ferrous bisglycinate will experience less of a gut pathogen expansion, in comparison to women receiving ferrous sulfate.

Project design

This study will be a sub-analysis of an in progress, CIHR funded 12-week double-blind, three-arm, placebo-controlled randomized controlled trial taking place in Cambodia. Participants were included if they were 18-45, female, apparently healthy, consented to participate in the study by providing stool and blood samples, and resided in the study location for the study period. Participants were excluded if they had any known illness or disease; were pregnant; or were taking antibiotics, non-steroidal anti-inflammatory drugs, dietary supplements, or vitamin and mineral supplements in the previous 12 weeks. Through parallel assignment, participants will either receive ferrous sulfate (60 mg daily oral pill), ferrous bisglycinate (18 mg daily oral pill), or placebo (daily oral pill of microcrystalline cellulose). Trial investigators, research staff, and participants will be blinded to the assigned interventions. Additional factors from participants will be analyzed such as serum ferritin and other biomarkers, and fecal calprotectin.

Objective

Compare beta-diversity and relative gut microbial taxonomic abundance between the three study arms at baseline and 12-weeks through 16S ribosome RNA (rRNA) sequencing.

Methods

Using fecal samples from a subset of 150 women (50 from each study arm at baseline and 12-weeks), DNA will be extracted, purified, and analyzed by 16S rRNA gene sequencing at the Integrated Microbiome Resource at Dalhousie University. Variable regions V6-V8 of the bacterial 16S rRNA gene will be amplified from the purified DNA and sequenced on an Illumina MiSeq using paired-end 300 bp sequencing (8). Analysis of sequencing data will be performed as described using Microbiome Helper (8). Raw data will be assessed for quality via FASTQC and paired-end reads will be stitched together using PEAR. Stitched reads will also be assessed for quality. VSERCH will be used to remove chimeric reads. Taxonomy will be assigned by matching sequences into operational taxonomic units with 97% sequence identity and comparing to the GreenGenes 16S rRNA database using QIIME. Sequencing depth will be normalized using DeSeq2's negative binomial distribution. Principle coordinate analysis will be conducted using UniFrac beta-diversity through QIIME.

Clinical significance

A growing body of literature supports the notion that iron supplementation can lead to disease by promoting expansion of enteric pathogens (9). In view of the fact that the WHO is promoting supplementation, there is a need to determine the potential for harm and if better alternatives are available. Results from this study will contribute to improving safety and effectiveness of iron supplementation for women worldwide.

Knowledge translation

Since commencing this project, we have published two review articles summarizing the effects of iron supplementation and fortification on the gut microbiota (10) and the impact of climate change on enteric pathogens in the Canadian Arctic (11). We aim to communicate the results of this project through a published manuscript and a presentation at a future AMMI CACMID national meeting.

References

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