Gut Microbiota and Inflammation in Patients which Schizophrenia

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Background and rationale

Recent research indicates that the gut microbiota and its associated microbiome play a critical role in regulating the host's metabolic and immune processes, particularly in relation to inflammation. This interaction occurs through the bidirectional communication within the gut-brain axis (GBA), involving the central nervous system (CNS) via the vagus nerve and the immune response pathways. According to Reale et al. (2011), microbial dysbiosis can lead to the production of both pro-inflammatory and anti-inflammatory interleukins, which participate in this reciprocal relationship, ultimately resulting in neuroinflammation that may contribute to the onset and exacerbation of symptoms associated with mental disorders, including schizophrenia.

The present study aims to elucidate the composition, taxonomy, and functional diversity of the intestinal microbiota by amplifying the genes of the hypervariable regions V6-V8, alongside the genera Bacteroides, Prevotella, and Porphyromonas, as well as the enzyme butyryl-CoA transferase. Furthermore, the inflammatory state will be assessed through the measurement of plasma concentrations of cytokines IL-1β, IL-6, IL-10, MCP-1, and TNF-α in male patients diagnosed with schizophrenia during the acute phase of their first psychotic episode, who are hospitalized in the Short Stay Unit of the “Dr. Samuel Ramírez Moreno” Psychiatric Hospital, compared to a control group of healthy individuals.

This integrative approach facilitates a comprehensive exploration of the interplay between intestinal microbiota and inflammation within the context of schizophrenia, thereby enhancing our understanding of the underlying mechanisms that contribute to this complex mental illness.

Objectives

The present study was aimed to identify the functional diversity of the intestinal microbiota and the inflammatory state in individuals with Schizophrenia diagnosed during their first attack of psychosis compared with healthy individuals.

1. Characterization of Gut Microbiota:
- To analyze the composition, taxonomy, and functional diversity of the intestinal microbiota in individuals diagnosed with schizophrenia during their first psychotic episode, focusing on the amplification of hypervariable regions V6-V8 and specific genera (Bacteroides, Prevotella, Porphyromonas).
2. Assessment of Inflammatory Markers:
- To evaluate the inflammatory state in schizophrenia patients by measuring plasma concentrations of key cytokines: IL-1β, IL-6, IL-10, MCP-1, and TNF-α.
3. Comparison with Healthy Controls:
- To compare the gut microbiota profiles and inflammatory markers between schizophrenia patients and a matched control group of healthy individuals, aiming to identify potential biomarkers associated with the inflammatory response in schizophrenia.
4. Understanding the Gut-Brain Axis:
- To explore the relationship between gut microbiota diversity and inflammatory cytokine levels, contributing to the understanding of the gut-brain axis and its implications for the pathophysiology of schizophrenia.

Methods

Study Design

This is a clinical, observational, cross-sectional, and prospective study.

- **Participants**

The study included 12 participants, comprising 4 individuals diagnosed with schizophrenia during their first psychotic episode and 8 healthy males serving as a control group. Inclusion criteria required that all participants were not on any medication that could influence inflammation or alter the intestinal microbiota. Participants were recruited from the Short Stay Unit of the “Dr. Samuel Ramírez Moreno” Psychiatric Hospital.

Data Collection

Demographic and clinical data were collected through structured interviews and medical history assessments, recorded in a case report form. Participants' ages were documented, with the schizophrenia group having a mean age of 31.25 ± 8.18 years and the control group 23.7 ± 5.67 years.

Methods

- **Microbiota Analysis**

The intestinal microbiota was characterized by amplifying the genes of the hypervariable regions V6-V8 using reverse transcription polymerase chain reaction (RT-PCR). The analysis specifically targeted the following genera:

- •Bacteroides
- •Prevotella
- •Porphyromonas

Additionally, the enzyme butyryl-CoA transferase was amplified to assess functional diversity.

- **Inflammatory State Assessment**

The inflammatory state of participants was evaluated by measuring plasma concentrations of the following cytokines using MILLIPLEX® luminescence technology (Luminex, USA):

- •IL-1β
- •IL-6
- •IL-10
- •MCP-1
- •TNF-α

- **Biochemical Assessments**

Biochemical parameters, including glucose levels and lipid profiles, were assessed to provide additional context regarding the metabolic state of participants. All biochemical and genetic analyses were conducted under controlled conditions to minimize confounding factors.

- **Statistical Analysis**

Statistical analyses were performed using appropriate software, with significance set at $p < 0.05$. Descriptive statistics were calculated for demographic, clinical, and biochemical variables. Comparisons between the schizophrenia group and the control group were conducted to assess differences in microbiota composition and inflammatory markers. Correlation analyses examined relationships between gut microbiota diversity and inflammatory cytokine levels.

Results

Between July and August 2021, a total of 25 participants were initially recruited, with 12 meeting the inclusion criteria for the study. Ultimately, 8 healthy controls were selected to maintain a 2:1 ratio with the 4 participants diagnosed with schizophrenia, based on body fat percentage (figure 1).

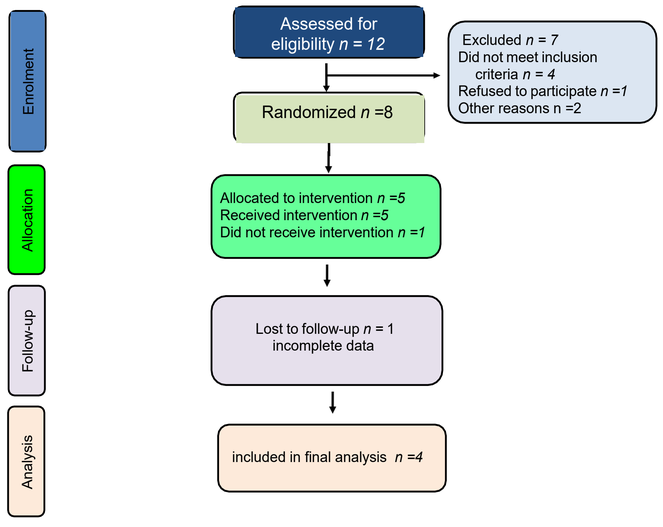

Figure 1. Flow of participants through the study stages, including eligibility, randomization, and final analysis.

References

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The study population consisted of 12 male participants, with an average age of 31.25 ± 8.18 years for the schizophrenia group and 23.7 ± 5.67 years for the control group. No statistically significant age difference was observed ($p = 0.90$). Anthropometric measurements (height, weight, BMI) showed no significant differences between groups ($p > 0.05$).

Biochemical Assessments

Biochemical parameters, including fasting serum concentrations of glucose, urea, creatinine, total cholesterol, triglycerides, and LDL, were comparable between groups ($p > 0.05$). However, HDL levels were significantly higher in the control group (50.13 mg/dL) compared to the schizophrenia group (31.50 mg/dL) ($p < 0.05$). Notably, triglyceride levels in the schizophrenia group (193.75 mg/dL) exceeded the recommended reference values (<150 mg/dL).

Intestinal Microbiota Analysis

DNA extraction methods indicated that samples treated with SSC yielded higher integrity than those processed with TRIzol. PCR results showed the presence of the bacterial genera Bacteroides, Prevotella, and Porphyromonas (figure 2). The hypervariable region V6-V8 was detected in 2 of 4 schizophrenia participants and 5 of 8 controls, with no significant difference between groups ($p = 0.692$). The mean Cq values for Bacteroides-Prevotella-Porphyromonas were lower in the schizophrenia group (22.17 ± 4.60) compared to controls (26.74 ± 8.39), suggesting greater amplification in the schizophrenia group, although this was not statistically significant , $p = 0.341$ (graphic 1).

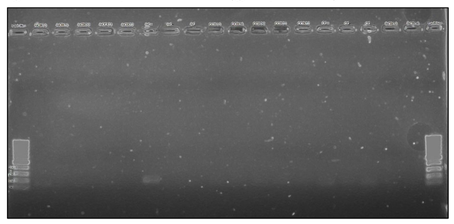
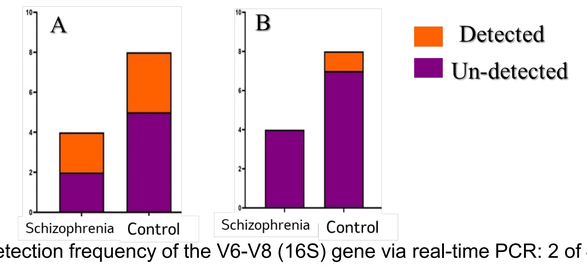


Figure 2. 1.5% Agarose Gel showing molecular weight markers (ladder) on both sides. Labels indicate: (A) PCR products for Bacteroides-Prevotella-Porphyromonas; (B) Hypervariable Region V6-V8; (C) Butyryl-CoA transferase. AC+ and CC+ are positive controls; AC- and CC- are negative controls.positive controls, while AC- and CC- represent negative controls.



Graphic 1:A) Detection frequency of the V6-V8 (16S) gene via real-time PCR: 2 of 4 schizophrenia participants amplified the gene, while 5 of 8 controls were detectable. B) Detection frequency of the Butyryl-CoA transferase gene via real-time PCR: detected in all schizophrenia participants (n=4) and in only 1 of 8 controls.

Cytokine Levels

Cytokine analysis revealed no detectable levels of IL-1β in either group. However, IL-10 concentrations were significantly higher in the schizophrenia group (14.80 ± 6.80 pg/mL) compared to controls (5.75 ± 2.57 pg/mL) ($p = 0.017$). IL-6 was detected in 3 of 4 schizophrenia participants, while none were detected in controls ($p = 0.018$). No significant differences were found for TNF-α ($p = 0.643$) or MCP-1 ($p = 0.089$).

Dietary Intake

Analysis of dietary intake showed no significant differences in the consumption of energy, proteins, lipids, carbohydrates, or fiber between groups. However, participants with schizophrenia exhibited limited dietary variability and higher daily caloric intake (4532 ± 1075 Kcal) compared to controls (3664 ± 1437 Kcal) ($p = 0.283$).

Conclusions

This study highlights significant differences in microbiota composition and inflammatory markers between schizophrenia patients and healthy controls, suggesting potential biomarkers for understanding the role of gut health in schizophrenia. Further research is needed to explore these associations and their implications for treatment.