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The Gut-Brain Axis: Investigating the Relationship Between Gut Microbiota Composition and Mental Health Disorders (Depression and Anxiety)

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Abstract

Aim: This study aimed to assess the association between gut microbiota composition and mental health symptoms in individuals diagnosed with depression and/or anxiety.

Methods: The study was a 12-month, observational cohort study with an interventional component involving participants diagnosed with depression and anxiety. Inclusion criteria included adults aged 18-65 who were diagnosed with moderate-to-severe depression or anxiety, based on DSM-5 criteria. Healthy controls had no current or previous diagnosis of psychiatric disorders. The random sampling was used to recruit 53 participants in each of the three strata (depression, anxiety, and healthy). The fecal samples were analyzed using 16S ribosomal RNA (rRNA) sequencing. To assess the severity of depression and anxiety symptoms, participants completed validated scales that included the Beck Depression Inventory (BDI) and the Hamilton Depression Rating Scale (HDRS) for those in the depression group. For participants with anxiety disorders, the Hamilton Anxiety Rating Scale (HAM-A) and the Generalized Anxiety Disorder (GAD-7) scale were employed.

Results: The study found that participants with depression and anxiety had lower levels of alpha diversity, as measured by the Shannon diversity index, compared to healthy controls. For example, the Shannon index for healthy controls averaged 4.5, while it was 3.2 and 3.1 for participants with depression and anxiety, respectively. This indicated reduced microbial diversity in individuals with mental health disorders. Participants with depression had an average BDI score of 28, indicating moderate-to-severe depression, while those with anxiety had an average HAM-A score of 25, reflecting moderate anxiety severity. After the 12-week intervention period, both the probiotic and dietary intervention groups showed significant reductions in symptom severity.

Recommendation: Clinicians should be trained to assess patients' gut health and recommend appropriate psychobiotic therapies, such as specific probiotic strains or high-fiber diets.

Keywords: *Gut microbiota, depression, anxiety, mental health, probiotic, diet.*

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INTRODUCTION

According to Mayer, Nance, and Chen (2022), the gut-brain axis refers to the bidirectional communication system between the gastrointestinal tract and the central nervous system (CNS), which plays a critical role in maintaining homeostasis and regulating physiological processes, including immune, neuroendocrine, and neural functions. The gut microbiota, a complex ecosystem of trillions of microorganisms residing in the gastrointestinal tract, has emerged as a central player in this interaction (Gomaa, 2020; Rea, Dinan & Cryan, 2020). Emerging evidence suggests that the gut microbiota can influence brain function through various pathways, including the production of neurotransmitters, modulation of the immune system, and production of short-chain fatty acids (SCFAs) that influence the permeability of the gut and blood-brain barrier (Anwar et al., 2021; Avelar Rodriguez,2019; Ahmed & Khan, 2023). Consequently, alterations in gut microbiota composition have been implicated in various psychiatric disorders, particularly depression and anxiety (Rea et al., 2020).

Mental health disorders such as depression and anxiety are among the leading causes of disability worldwide, contributing to substantial societal and economic burdens (Santomauro et al., 2021; Rehm & Shield, 2019). Despite advances in pharmacological treatments, a significant proportion of patients fail to respond adequately to conventional therapies. This has led to an increasing interest in exploring alternative therapeutic strategies, including those targeting the gut microbiota. Recent studies have demonstrated that individuals with mental health disorders, particularly depression and anxiety, tend to exhibit significant alterations in their gut microbial composition compared to healthy individuals (Peirce & Alviña, 2019; Huang, 2019; Audet, 2019). Specifically, reduced microbial diversity and the overrepresentation of pathogenic bacteria, such as Clostridium, Enterobacteriaceae, and Bacteroides, have been observed in these populations.

Rationale for the Study

Despite growing evidence of the relationship between gut microbiota and mental health, many aspects of this relationship remain under researched. Existing studies often vary in methodology, population size, and microbiota assessment techniques, leading to inconsistent findings. Furthermore, the specific microbial taxa involved in mental health disorders, the pathways through which they exert their effects, and the role of dietary interventions in modulating these effects require further exploration. This study aims to address these gaps by conducting a comprehensive investigation into the gut microbiota composition in individuals with depression and anxiety, compared to healthy controls. Additionally, it will explore whether interventions aimed at improving gut health can positively influence mental health outcomes.

The results of this research could have significant clinical implications by providing a deeper understanding of the gut-brain axis and identifying novel, non-pharmacological treatment strategies for depression and anxiety. If a strong association between gut microbiota and mental health is confirmed, it may pave the way for the development of "psychobiotics" — probiotics and dietary interventions specifically designed to target mental health disorders.

Research Objectives

Primary Objective: To assess the association between gut microbiota composition and mental health symptoms in individuals diagnosed with depression and/or anxiety.

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Secondary Objective: To evaluate changes in gut microbiota after interventions aimed at improving gut health (e.g., probiotics, dietary changes) and their impact on mental health outcomes.

Research Questions

1. Is there a significant difference in the gut microbiota diversity of individuals with depression or anxiety compared to healthy controls?
2. Are specific microbial species or compositions associated with the severity of depressive or anxiety symptoms?
3. Can interventions targeting gut health (probiotics, dietary changes) improve mental health outcomes in individuals with depression and/or anxiety?

METHODOLOGY

Study Type

The study was a 12-month, observational cohort study with an interventional component involving participants diagnosed with depression and anxiety. A 12-month period allowed for longitudinal assessment of microbiota changes and mental health outcomes, including pre- and post-intervention evaluations for the interventional group. The observational component assessed baseline microbiota composition and mental health status in participants with depression, anxiety, and healthy controls. The interventional component evaluated the impact of probiotic supplementation and dietary interventions on gut microbiota composition and mental health outcomes in participants with depression or anxiety.

Inclusion and Exclusion Criteria

Inclusion criteria included adults aged 18-65 who were diagnosed with moderate-to-severe depression or anxiety, based on DSM-5 criteria. Healthy controls had no current or previous diagnosis of psychiatric disorders. Exclusion criteria included individuals with chronic gastrointestinal conditions, those currently using antibiotics or probiotics, individuals with severe physical health conditions that could affect microbiota composition, and pregnant or breastfeeding individuals.

Sample Size

The stratified sampling approach was used to ensure recruitment of an equal number of participants across the three groups. The stratification was based on mental health status (depression, anxiety, and healthy), allowing for direct comparison across these groups. After stratifying by diagnosis, participants were randomly selected from those who meet the inclusion criteria. This approach minimized selection bias and ensures that the sample represents a cross-section of the population relevant to the study's objectives. The total sample size of 153 participants was determined based on power analysis to ensure sufficient statistical power to detect differences between groups. Each group (depression, anxiety, and healthy control) included 51 participants, which is sufficient for performing comparative analyses while controlling for potential confounders like age, sex, diet, and lifestyle.

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Data Collection Methods

Gut Microbiota Analysis

Fecal samples were collected from all participants as part of the gut microbiota analysis. Each participant provided a sample in sterile containers, which were subsequently stored under controlled conditions to preserve microbial viability. The samples were then analyzed using 16S ribosomal RNA (rRNA) sequencing. This method involved extracting DNA from the fecal samples, amplifying the 16S rRNA gene regions specific to bacteria, and sequencing them to generate detailed profiles of microbial composition. The resulting data provided insights into the diversity and richness of gut microbiota across different participant groups, enabling comparisons between those diagnosed with depression, anxiety, and healthy controls. This analysis was crucial for understanding the potential links between gut microbiota and mental health outcomes.

Psychological Assessments

To assess the severity of depression and anxiety symptoms, standardized self-report questionnaires and clinician-administered assessments were utilized. Participants completed validated scales that included the Beck Depression Inventory (BDI) and the Hamilton Depression Rating Scale (HDRS) for those in the depression group. For participants with anxiety disorders, the Hamilton Anxiety Rating Scale (HAM-A) and the Generalized Anxiety Disorder (GAD-7) scale were employed. These assessments were administered both at baseline and following the intervention period, allowing for a comprehensive evaluation of changes in mental health status. The use of these standardized instruments ensured that symptom severity was measured consistently across participants, facilitating reliable comparisons and analyses of mental health outcomes (Costa et al., 2021).

Dietary Analysis

Participants' dietary habits were evaluated using food frequency questionnaires (FFQs) designed to capture detailed information about their usual food intake. The FFQs focused on key dietary components, such as fiber intake, processed food consumption, and overall dietary patterns. Participants were asked to report the frequency and portion sizes of various food items consumed over a specified period, providing valuable data on their eating habits. This dietary information was crucial for analyzing the relationship between nutrition and gut microbiota composition, as it allowed researchers to assess how dietary patterns influenced microbial diversity and potentially affected mental health outcomes. Changes in dietary intake during the intervention period were monitored, enabling a thorough understanding of how dietary modifications might correlate with shifts in both gut health and psychological well-being.

Study Setting

The study was conducted in collaboration with a clinical research institute and a laboratory specializing in microbiota analysis. Fecal sample collection and psychological assessments occured at regular intervals in a clinical setting.

Phase 1: Baseline Assessment

During the baseline phase, participants provided fecal samples for microbiota analysis. These samples were stored under sterile conditions and analyzed using 16S ribosomal RNA (rRNA) sequencing. This method identified the microbial species present in the samples and assessed the

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diversity and richness of each participant's microbiota. Previous research indicated that gut dysbiosis, an imbalance in gut microbiota, was linked to psychiatric disorders such as depression and anxiety (Borrego-Ruiz & Borrego, 2024).

Psychological assessments were conducted to evaluate the severity of depressive and anxiety symptoms. Participants in the depression group were assessed using the Beck Depression Inventory (BDI) and the Hamilton Depression Rating Scale (HDRS), while those in the anxiety group were assessed using the Hamilton Anxiety Rating Scale (HAM-A) and the Generalized Anxiety Disorder (GAD-7) scale. Healthy control participants underwent psychological evaluations to confirm the absence of any psychiatric disorders, ensuring that they exhibited no signs of depression or anxiety.

These validated scales provided a standardized method for quantifying the severity of depression and anxiety, allowing for consistency in mental health research (Breedvelt, 2020). In addition to psychological assessments, participants completed detailed dietary questionnaires that documented their intake of key nutrients, especially fiber, prebiotics, and processed foods. Lifestyle factors such as physical activity, sleep patterns, alcohol consumption, and smoking were also recorded using standardized tools, given their known influence on gut microbiota composition (Pedroza Matute & Iyavoo, 2023).

Phase 2: Intervention Component

In the intervention phase, a subgroup of participants from the depression and anxiety groups were randomized into one of three intervention groups, where they participated for a 12-week period. Group A received a daily probiotic supplement containing bacterial strains known to affect the gut-brain axis, such as *Lactobacillus* and *Bifidobacterium*. Previous studies suggested that probiotics could enhance gut microbiota composition and reduce inflammation, potentially improving mental health outcomes (Methiwala et al., 2021). Group B followed a high-fiber diet rich in prebiotic foods such as fruits, vegetables, and whole grains, designed to promote the growth of beneficial gut bacteria. It had been demonstrated that an increased intake of fiber could significantly enhance microbial diversity and improve gut health (Cronin et al., 2021). Group C served as the control group and received no dietary or probiotic intervention. These participants continued with their usual diet and lifestyle routines. This control group allowed for comparison to evaluate the natural progression of depression and anxiety symptoms without any microbiota-targeted interventions.

Participants in the intervention groups were regularly monitored to ensure compliance with the probiotic and dietary protocols. Adherence was assessed through self-reports and periodic interviews, ensuring that participants followed their assigned interventions throughout the study.

Phase 3: Follow-up and Post-intervention Assessment

After the 12-week intervention period, fecal samples were recollected from all participants. These post-intervention samples were analyzed using the same 16S rRNA sequencing technique to compare microbiota composition before and after the interventions. Shifts in microbial diversity, richness, or specific bacterial species were examined to assess the impact of the interventions. In addition to fecal sample collection, participants in all groups underwent the same psychological assessments used during the baseline phase (BDI, HDRS, HAM-A, and GAD-7). The goal was to measure changes in symptom severity following the intervention. Any changes in mental health

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status were then correlated with changes in gut microbiota to explore potential causal relationships between alterations in microbiota and improvements (or deterioration) in mental health.

RESULTS

Primary Outcomes

Gut Microbiota Diversity and Composition

One of the primary outcomes involved evaluating differences in gut microbiota diversity and composition between participants diagnosed with depression, anxiety, and healthy controls. Baseline fecal samples were analyzed using 16S rRNA sequencing to determine microbial richness and diversity. The study found that participants with depression and anxiety had lower levels of alpha diversity, as measured by the Shannon diversity index, compared to healthy controls. For example, the Shannon index for healthy controls averaged 4.5, while it was 3.2 and 3.1 for participants with depression and anxiety, respectively. This indicated reduced microbial diversity in individuals with mental health disorders.

Following the probiotic and dietary interventions, changes in microbiota composition were assessed to determine the efficacy of these interventions in altering gut health. Participants in the probiotic group experienced an increase in alpha diversity, with their post-intervention Shannon index rising by an average of 0.8 points, while the dietary intervention group showed an increase of 0.6 points. These findings suggested that both interventions positively influenced microbial diversity, with probiotics showing slightly stronger effects. Furthermore, specific bacterial species associated with improved gut-brain communication, such as Lactobacillus and Bifidobacterium, increased in abundance in the intervention groups.

Mental Health Symptoms

Mental health symptoms were assessed through standardized scales, including the Beck Depression Inventory (BDI), Hamilton Depression Rating Scale (HDRS), Hamilton Anxiety Rating Scale (HAM-A), and Generalized Anxiety Disorder (GAD-7). At baseline, participants with depression had an average BDI score of 28, indicating moderate-to-severe depression, while those with anxiety had an average HAM-A score of 25, reflecting moderate anxiety severity. After the 12-week intervention period, both the probiotic and dietary intervention groups showed significant reductions in symptom severity.

Participants in the probiotic group with depression experienced an average reduction of 9 points in their BDI score, bringing it to 19, which reflected mild depression. Those in the dietary intervention group also showed improvements, with an average reduction of 7 points, bringing their score to 21. Similarly, anxiety participants in the probiotic group saw their HAM-A scores drop by an average of 8 points, while the dietary intervention group exhibited a 6-point reduction. These reductions in mental health symptoms suggested a positive correlation between improved gut health and mental well-being.

Secondary Outcomes

Influence of Dietary Patterns

The study also explored the relationship between habitual dietary patterns and gut microbiota composition. At baseline, participants with higher fiber intake showed greater alpha diversity, with

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an average Shannon index of 4.0, compared to 3.1 for those with lower fiber consumption. Participants who habitually consumed high amounts of processed foods had reduced microbial diversity, with a Shannon index averaging 2.9. During the intervention, participants in the dietary group who adhered to the high-fiber diet showed significant improvements in their gut microbiota, with the Shannon index increasing by 0.6 points on average, while those in the probiotic group showed a similar trend. This demonstrated the strong influence of diet on gut health, highlighting fiber's role in enhancing microbial diversity and potentially improving mental health outcomes.

Inflammatory Markers

Blood samples were collected to assess systemic inflammation, as inflammation has been suggested as a mediator between gut health and mental disorders. At baseline, participants with depression and anxiety had elevated levels of C-reactive protein (CRP) and interleukin-6 (IL-6), with CRP averaging 3.8 mg/L and IL-6 averaging 5.5 pg/mL in these groups. Healthy controls, in contrast, had CRP levels averaging 1.2 mg/L and IL-6 levels of 1.8 pg/mL.

Following the probiotic and dietary interventions, reductions in inflammatory markers were observed in both groups. Participants in the probiotic group experienced a decrease in CRP by 1.5 mg/L and IL-6 by 2.0 pg/mL, while the dietary group saw reductions of 1.2 mg/L and 1.7 pg/mL, respectively. These results indicated that improving gut microbiota through dietary or probiotic means could help lower systemic inflammation, potentially contributing to improved mental health outcomes.

Statistical Analysis

Descriptive Statistics

Baseline characteristics, including demographic data, gut microbiota diversity, and psychological assessments, were summarized using descriptive statistics. For example, the average age of participants was 35 years, with even distribution between men and women. Microbiota diversity at baseline was summarized using the Shannon diversity index, while psychological assessments were reported as mean scores for each group. The average BDI score for the depression group was 28, while the anxiety group had an average HAM-A score of 25.

Microbiota Analysis

To analyze gut microbiota diversity, alpha and beta diversity metrics were calculated. Alpha diversity, reflecting within-group diversity, was measured using the Shannon index, while beta diversity, representing between-group differences, was measured using Bray-Curtis dissimilarity. Comparisons were made using ANOVA. Results showed significant differences in baseline microbiota composition between the depression, anxiety, and healthy control groups, with p-values < 0.05 indicating statistically significant differences.

Intervention Effects

To assess the effects of the probiotic and dietary interventions on microbiota and mental health outcomes, repeated measures ANOVA and mixed-effects models were employed. These analyses allowed for the evaluation of changes over time, comparing baseline and post-intervention data across groups. Both the probiotic and dietary intervention groups showed statistically significant improvements in both gut microbiota diversity and mental health symptoms compared to the

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control group, with p-values < 0.01 for reductions in BDI and HAM-A scores. Mixed-effects models controlled for potential confounders such as age, diet, and physical activity.

CONCLUSION

This study aims to uncover the complex interactions between gut health and mental health, providing evidence that could lead to more holistic and personalized approaches to treating psychiatric disorders. If a link between gut health and mental well-being is established, this may encourage further research into psychobiotics and dietary modifications as viable treatments for depression and anxiety.

The findings from this study contributed significantly to the expanding field of psychobiotics and the gut-brain axis, advancing the understanding of the relationship between gut health and mental disorders such as depression and anxiety. By investigating the composition and diversity of gut microbiota in individuals with these mental health conditions and healthy controls, the study provided new insights into how gut dysbiosis, a disruption in the balance of gut microbes, may be linked to psychological distress. The identification of specific microbial species associated with improved or worsened mental health symptoms offered valuable evidence to support the theory that gut microbiota plays a pivotal role in influencing mental well-being.

The results also highlighted the potential for non-pharmacological interventions in the treatment of mental health disorders. Both probiotic supplementation and dietary interventions showed promising outcomes, with significant improvements in both gut microbiota diversity and reductions in depressive and anxiety symptoms. These findings indicated that altering gut health through diet and probiotic intake could serve as an adjunct or alternative to conventional pharmacological treatments, which often come with side effects and may not be effective for all individuals.

RECOMMENDATIONS

1. Clinicians should be trained to assess patients' gut health and recommend appropriate psychobiotic therapies, such as specific probiotic strains (e.g., Lactobacillus, Bifidobacterium) or high-fiber diets.
2. Researchers should continue investigating which probiotic strains and dietary components are most effective in improving mental health outcomes, refining these therapies over time.
3. Pharmaceutical companies could invest in developing clinically validated probiotic supplements targeted at mental health conditions, ensuring quality and effectiveness.
4. Clinicians should collaborate with laboratories to conduct gut microbiota profiling of patients diagnosed with depression and anxiety. Based on this profile, targeted probiotic or dietary recommendations could be made.
5. Policy Makers and health administrators should enact policies to ensure that microbiota profiling becomes an affordable and widely available service for patients, integrating it into healthcare systems.
6. Medical schools and training institutions should update curricula to include the latest research on the gut-brain axis, teaching future healthcare professionals about psychobiotics and their role in mental health care.

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7. Media outlets and mental health advocacy organizations could spread awareness about the connection between gut health and mental health, encouraging individuals to adopt gut-friendly diets.
8. Mental health services should include dietitians as part of their care teams to provide patients with dietary assessments and tailored nutritional advice.
9. The food industry should be encouraged to develop and market products that support gut health, such as high-fiber, prebiotic, and probiotic-enriched foods.

Ethical Considerations

Ethical considerations were rigorously addressed throughout the study to ensure participant safety, autonomy, and compliance with ethical standards. Prior to the initiation of any study procedures, informed consent was obtained from all participants. Everyone was thoroughly informed about the study's objectives, the nature of the interventions, and any potential risks or benefits involved. This ensured that participants fully understood their role in the research and agreed voluntarily to take part. In addition, participants were explicitly told about their right to withdraw from the study at any time without penalty or loss of benefits, safeguarding their autonomy and well-being.

Ethical Approval

The study also underwent careful ethical review to secure approval from the institutional review board (IRB). This process involved submitting a detailed protocol outlining the study design, procedures, potential risks, and strategies to mitigate any harm to participants. The IRB assessed the study to ensure it adhered to established ethical guidelines, particularly concerning human subjects' research, and granted approval before data collection commenced.

Participant Safety

Specific steps were taken to ensure that the collection of fecal samples was performed in a manner that prioritized participant comfort and safety. Standard hygienic protocols were followed during the collection, handling, and storage of samples, minimizing any risk of contamination or infection. Participants were provided with clear instructions on how to safely collect and submit their samples, and all materials used were sterile and disposed of according to biosafety guidelines. These procedures ensured both participant safety and the integrity of the biological data collected.

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