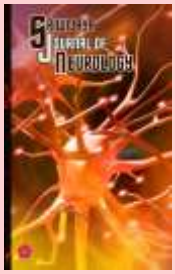

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Gut-Brain Axis Dysfunction in Parkinson's Disease: Early Biomarkers and Therapeutic Potential in Jakarta, Indonesia

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A B S T R A C T

Introduction: Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor and non-motor symptoms. Increasing evidence suggests a crucial role of gut-brain axis dysfunction in PD pathogenesis. This study aimed to investigate the gut microbiome composition, identify potential early biomarkers, and explore the therapeutic potential of targeting the gut-brain axis in PD patients in Jakarta, Indonesia. **Methods:** This cross-sectional study involved 50 PD patients and 50 age-matched healthy controls from Jakarta. Stool and blood samples were collected for 16S rRNA gene sequencing to analyze gut microbiome composition, and serum inflammatory markers (TNF-α, IL-6, and CRP) were measured using ELISA. Clinical data, including disease duration, severity, and non-motor symptoms, were assessed using standardized scales. **Results:** PD patients exhibited significant alterations in gut microbiome composition compared to controls, with a decrease in beneficial bacteria (e.g., *Faecalibacterium prausnitzii*) and an increase in pro-inflammatory bacteria (e.g., *Enterobacteriaceae*). Elevated levels of serum inflammatory markers were also observed in PD patients. Correlation analysis revealed associations between specific gut microbiota, inflammatory markers, and disease severity. **Conclusion:** This study provides evidence of gut-brain axis dysfunction in PD patients in Jakarta, Indonesia. Alterations in gut microbiome composition and increased systemic inflammation may serve as potential early biomarkers and therapeutic targets for PD. Further research is needed to explore the causal relationship and develop targeted interventions.

1. Introduction

Parkinson's disease (PD) is a prevalent neurodegenerative disorder, second only to Alzheimer's disease, with a global impact on millions of individuals. The disease is primarily characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, a region of the brain crucial for motor control. This neuronal loss leads to a range of motor symptoms, the hallmark features of PD, including bradykinesia (slowness of movement), rigidity (muscle stiffness), tremor (involuntary shaking), and postural instability (difficulty maintaining balance). While these motor symptoms dominate the clinical picture of PD, the disease is also marked by a diverse array of non-motor symptoms (NMS). These NMS encompass a broad

spectrum of manifestations, including gastrointestinal dysfunction (such as constipation and impaired gastric motility), sleep disturbances (like insomnia and REM sleep behavior disorder), cognitive decline (ranging from mild cognitive impairment to dementia), and mood disorders (such as depression and anxiety). The impact of NMS on the quality of life of PD patients is substantial, often even exceeding that of the motor symptoms. Moreover, NMS can precede the onset of motor symptoms by several years, suggesting a potential role in the early stages of PD pathogenesis. The underlying causes of PD are complex and multifactorial, with a combination of genetic predisposition, environmental factors, and the aging process playing significant roles. In recent years, the gut-brain axis has emerged as a crucial player in the development and progression of PD. This bidirectional communication system links the central nervous system (CNS) with the enteric nervous system (ENS), which governs the gastrointestinal tract. The gut-brain axis is a complex network involving neural pathways (such as the vagus nerve), endocrine signaling (via hormones and neurotransmitters), and immune mechanisms (involving gut-associated lymphoid tissue and inflammatory mediators).[1-4]

At the heart of the gut-brain axis lies the gut microbiome, a vast and dynamic community of trillions of microorganisms residing in the human gastrointestinal tract. These microorganisms, including bacteria, fungi, viruses, and archaea, play a critical role in human health and disease. The gut microbiome influences various physiological processes, including nutrient metabolism, immune system development, and even brain function. In the context of PD, research has revealed significant alterations in the gut microbiome composition of patients compared to healthy individuals. These alterations, often referred to as gut dysbiosis, are characterized by a decrease in beneficial bacteria (such as those producing short-chain fatty acids like butyrate) and an increase in potentially harmful or pro-inflammatory bacteria. This dysbiosis can disrupt the delicate balance of the gut-brain axis, contributing to the pathogenesis of PD. One of the key mechanisms by which gut dysbiosis is thought to influence PD is through increased intestinal permeability, often referred to as "leaky gut." The intestinal lining acts as a barrier, controlling the passage of substances between the gut and the bloodstream. Gut dysbiosis can compromise this barrier, allowing bacterial metabolites and toxins to enter the systemic circulation, triggering inflammation throughout the body. This systemic inflammation is believed to contribute to neuroinflammation, a chronic inflammatory state in the brain that is increasingly recognized as a critical factor in neurodegenerative diseases like PD.[5-7]

Moreover, gut microbiome dysbiosis can also impact the production and regulation of neurotransmitters, including dopamine and serotonin, which are essential for motor control, mood regulation, and cognitive function. Certain gut bacteria are involved in the synthesis of dopamine precursors, and alterations in their abundance may influence dopamine levels in the brain. Given the central role of dopamine deficiency in PD, this gut-brain interaction has significant implications for disease progression. The identification of early biomarkers for PD is crucial for timely diagnosis and the development of effective interventions. Traditional diagnostic approaches for PD rely primarily on clinical assessment of motor symptoms, which often manifest after significant neuronal loss has already occurred. The emerging role of the gut-brain axis in PD pathogenesis has highlighted the potential of gut microbiome composition and related metabolites as early biomarkers. Changes in the gut microbiome may precede the onset of motor symptoms, providing a window of opportunity for early intervention and disease modification. Furthermore, the gut-brain axis offers a promising target for novel therapeutic strategies in PD. Targeting the gut microbiome through dietary interventions, prebiotics (non-digestible food ingredients that promote the growth of beneficial bacteria), probiotics (live microorganisms that confer health benefits), or fecal microbiota transplantation (transfer of fecal matter from a healthy donor to a recipient) may hold therapeutic potential. These approaches aim to restore gut microbiome balance, reduce inflammation, and potentially slow

the progression of PD.[8-10] This study focuses on investigating the gut microbiome composition, identifying potential early biomarkers, and exploring the therapeutic potential of targeting the gut-brain axis in PD patients in Jakarta, Indonesia.

2. Methods

This research employed a cross-sectional design, involving a single assessment of participants at a specific point in time. The study was conducted at the Neurology Clinic of a tertiary hospital in Jakarta, Indonesia, over a period of one year, from January 2022 to December 2022. The study protocol was reviewed and approved by the CMHC Indonesia ethics committee, ensuring adherence to ethical guidelines for research involving human subjects. All participants provided written informed consent before enrollment in the study, indicating their voluntary participation and understanding of the study procedures. The study involved two groups of participants; Parkinson's Disease Patients: A total of 50 individuals diagnosed with idiopathic Parkinson's disease (PD) according to the UK Brain Bank criteria were included in the study; Healthy Controls: A group of 50 age-matched healthy individuals with no history of neurological or gastrointestinal disorders served as the control group. To minimize potential confounding factors, exclusion criteria were applied to both groups. Participants were excluded if they had used antibiotics or probiotics within the three months preceding the study, had a diagnosis of inflammatory bowel disease, or had other chronic diseases that could influence the gut microbiome or inflammatory markers.

Comprehensive data collection was performed to gather relevant information about the participants. This included; Demographic Data: Questionnaires were used to collect demographic information, including age, sex, and body mass index (BMI); Clinical Data: A neurologist conducted standardized assessments to gather clinical data, including disease duration, disease severity (assessed using the Hoehn and Yahr scale), and non-motor symptoms (evaluated using the Non-Motor Symptoms Scale).

Biological samples were collected from all participants to analyze gut microbiome composition and inflammatory markers; Stool Samples: Participants provided stool samples using sterile containers, following standardized procedures to minimize contamination. The collected samples were immediately stored at -80°C to preserve the integrity of the microbial DNA; Blood Samples: Blood samples were collected from participants in the morning after an overnight fast to minimize the influence of food intake on blood parameters. Serum was separated from the blood samples by centrifugation and stored at -80°C until analysis.

The analysis of the gut microbiome involved a multi-step process; DNA Extraction: Microbial DNA was extracted from the stool samples using a commercially available DNA extraction kit (Qiagen, Hilden, Germany), following the manufacturer's instructions; 16S rRNA Gene Amplification: The V3-V4 region of the 16S rRNA gene, a widely used marker for bacterial identification, was amplified using polymerase chain reaction (PCR) with specific primers designed to target this region; Sequencing: The amplified PCR products were sequenced using the Illumina MiSeq platform (Illumina, San Diego, CA, USA), a high-throughput sequencing technology that generates millions of DNA sequence reads; Data Processing: The raw sequencing data obtained from the Illumina MiSeq platform were processed using QIIME2 software, a bioinformatics pipeline specifically designed for microbiome analysis. This processing included quality filtering to remove low-quality reads, denoising to correct sequencing errors, and chimera removal to eliminate artificial sequences; OTU Clustering: The processed sequences were clustered into operational taxonomic units (OTUs) based on 97% similarity, representing groups of closely related bacteria; Diversity Analysis: Alpha diversity analysis was performed to assess the microbial diversity within each sample, using metrics such as the number of observed OTUs, Shannon index, and Simpson index. Beta diversity analysis was conducted to evaluate the differences in microbial community structure between samples, using Bray-Curtis dissimilarity and Jaccard distance metrics; Taxonomic Classification: The representative sequences of the OTUs were taxonomically classified using the SILVA database, a

comprehensive database of ribosomal RNA sequences, to identify the bacterial taxa present in the samples.

The levels of inflammatory markers in the serum samples were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). The markers measured included; Tumor Necrosis Factor-alpha (TNF-α): A pro-inflammatory cytokine involved in systemic inflammation; Interleukin-6 (IL-6): Another pro-inflammatory cytokine that plays a role in immune regulation and inflammation; C-reactive Protein (CRP): An acute-phase protein produced by the liver in response to inflammation. The ELISA assays were performed according to the manufacturer's instructions, ensuring accurate and reliable measurement of the inflammatory markers.

Statistical analysis of the collected data was performed using SPSS software (IBM, Armonk, NY, USA). The specific statistical tests used depended on the type of data being analyzed; Continuous Variables: Continuous variables, such as age, BMI, and inflammatory marker levels, were presented as mean ± standard deviation (SD) if they followed a normal distribution, or as median (interquartile range) if they did not. Differences between PD patients and controls were assessed using Student's t-test for normally distributed data or the Mann-Whitney U test for non-normally distributed data; Categorical Variables: Categorical variables, such as sex, were presented as frequencies and percentages. Differences between groups were analyzed using the chi-square test or Fisher's exact test, depending on the sample size. Correlation analysis was performed to investigate the relationships between gut microbiota abundance, inflammatory marker levels, and disease severity. Spearman's rank correlation coefficient was used to assess these correlations, as it is a non-parametric test that does not assume a linear relationship between variables. For all statistical tests, a p-value of less than 0.05 was considered statistically significant, indicating that the observed differences or correlations were unlikely to occur by chance alone.

3. Results

Table 1 presents the demographic and clinical characteristics of the 50 Parkinson's Disease (PD) patients and 50 healthy controls participating in the study; Age and Sex: Both groups were carefully matched in terms of age and sex distribution, ensuring that these factors did not confound the comparisons between the groups. The average age was approximately 62 years in both groups, with a fairly even distribution of males and females. This matching is crucial because age and sex can influence both gut microbiome composition and inflammatory responses; BMI: The Body Mass Index (BMI) was also similar between the PD patients and healthy controls, suggesting that differences in body weight were unlikely to significantly impact the study findings; Disease Characteristics: The table provides information specific to the PD patients, including the average disease duration (4.8 years), the typical disease severity as measured by the Hoehn and Yahr scale (stage 2), and the median score on the Non-Motor Symptoms Scale (12). These details help characterize the PD patient group and provide context for interpreting the results related to gut microbiome composition and inflammation; Statistical Significance: The p-values in the table indicate that there were no statistically significant differences between the PD patients and healthy controls in terms of age, sex, or BMI. This confirms the successful matching of the two groups for these key demographic factors.

Table 1. Demographic and clinical characteristics of participants.

Characteristic	PD Patients (n=50)	Healthy Controls (n=50)	P-value
Age (years)	62.5 ± 8.3	61.2 ± 7.9	0.412
Gender (male/female)	28/22	30/20	0.785
BMI (kg/m^2)	24.8 ± 3.5	24.2 ± 3.1	0.357
Disease Duration (years)	4.8 (2.5-7.1)	-	-
Hoehn and Yahr Stage	2 (1-3)	-	-
Non-Motor Symptoms Scale Score	12 (8-16)	-	-

*Data are presented as mean ± SD, median (interquartile range), or number (%).

Table 2 presents the analysis of gut microbiome diversity in PD patients and healthy controls from Jakarta, considering both alpha and beta diversity. Alpha diversity measures the variety of bacterial species within each individual's gut. The table shows three different metrics for alpha diversity; Observed OTUs: This represents the number of different bacterial species (or closely related groups of bacteria) identified in each sample. PD patients had significantly fewer observed OTUs (350 ± 80) compared to healthy controls (420 ± 95), as indicated by the low p-value (0.002). This suggests a reduced richness of bacterial species in the gut of PD patients; Shannon Index: This index takes into account both the number of species and their relative abundance. The Shannon index was also significantly lower in PD patients (5.8 ± 1.2) compared to controls (6.5 ± 1.4), with a p-value of 0.015. This further supports the finding of lower bacterial diversity in PD patients; Simpson Index: This index gives more weight to the abundance of dominant species. Similar to the other two metrics, the Simpson index was significantly lower in PD patients (0.88 ± 0.05) than in controls (0.92 ± 0.04), with a p-value of 0.008. This indicates that the gut microbiome of PD patients may be dominated by a few bacterial species, with less evenness in the distribution of different species. Taken together, the alpha diversity measures consistently show a significant reduction in the diversity of gut bacteria in PD patients compared to healthy controls. This reduced diversity could have implications for gut health and overall well-being, as a diverse microbiome is generally considered to be more resilient and beneficial. Beta diversity measures the differences in bacterial community composition between individuals or groups. The table shows two metrics for beta diversity; Bray-Curtis Dissimilarity: This metric quantifies the dissimilarity between two samples based on the abundance of different species. A higher value indicates greater dissimilarity. While the table doesn't provide a p-value for this metric, the average Bray-Curtis dissimilarity (0.65 ± 0.10) suggests a substantial difference in bacterial community composition between PD patients and controls; Jaccard Distance: This metric focuses on the presence or absence of species, rather than their abundance. Similar to Bray-Curtis dissimilarity, the Jaccard distance (0.78 ± 0.12) indicates a considerable difference in the types of bacteria present in the gut of PD patients compared to controls.

Table 2. Alpha and beta diversity of gut microbiome in PD patients and healthy controls in Jakarta.

Diversity measure	**PD patients (n=50)**	**Healthy controls (n=50)**	**P-value**
Alpha diversity			
Observed OTUs	350 ± 80	420 ± 95	0.002
Shannon index	5.8 ± 1.2	6.5 ± 1.4	0.015
Simpson index	0.88 ± 0.05	0.92 ± 0.04	0.008
Beta diversity			
Bray-Curtis dissimilarity	0.65 ± 0.10	-	-
Jaccard distance	0.78 ± 0.12	-	-

Table 3 provides a detailed look at the relative abundance of key gut microbiota at the genus level in the PD patients and healthy controls participating in the study in Jakarta. This table highlights some crucial differences in the gut bacterial composition between these two groups; *Faecalibacterium:* This genus, known for its anti-inflammatory properties and production of butyrate (a short-chain fatty acid beneficial for gut health), was significantly lower in PD patients (8.5 ± 3.2%) compared to controls (12.8 ± 4.5%). This finding aligns with previous research suggesting a depletion of beneficial bacteria in PD; *Prevotella:* Another genus often associated with beneficial effects, *Prevotella*, was also less abundant in PD patients (15.3 ± 5.1%) compared to controls (21.7 ± 6.8%); *Akkermansia:* This genus, known for its role in maintaining gut barrier integrity and improving metabolic health, was found in lower amounts in PD

patients (2.1 ± 1.8%) compared to controls (3.9 ± 2.5%); *Bifidobacterium:* A genus often used in probiotic supplements due to its positive effects on gut health, *Bifidobacterium* was also less abundant in the PD group (1.8 ± 1.2%) compared to controls (3.2 ± 1.9%); *Lactobacillus:* Similarly, *Lactobacillus*, another common probiotic genus, was less prevalent in PD patients (0.8 ± 0.5%) than in controls (1.5 ± 0.8%); *Escherichia/Shigella:* This genus includes bacteria that can be pathogenic and contribute to inflammation. PD patients showed a higher abundance of *Escherichia/Shigella* (10.2 ± 4.1%) compared to controls (6.5 ± 2.8%). This increase could potentially contribute to gut inflammation and influence the gut-brain axis in PD; *Bacteroides:* This genus was more abundant in PD patients (28.5 ± 7.9%) compared to controls (24.3 ± 6.1%). While *Bacteroides* species can have both beneficial and detrimental effects, the implications of this increase in the context of PD require further investigation; *Ruminococcus:* This genus showed a slight increase in PD patients (14.6 ± 5.3%) compared to controls (11.2 ± 4.2%), but the difference was not as pronounced as for other genera; *Enterococcus:* There was no significant difference in the abundance of *Enterococcus* between PD patients (3.5 ± 2.1%) and controls (2.8 ± 1.5%).

Table 3. Relative abundance of key gut microbiota at the genus level in PD patients and healthy controls in Jakarta.

Genus	PD Patients (n=50) (%)	Healthy Controls (n=50) (%)
Faecalibacterium	8.5 ± 3.2	12.8 ± 4.5
Prevotella	15.3 ± 5.1	21.7 ± 6.8
Akkermansia	2.1 ± 1.8	3.9 ± 2.5
Bacteroides	28.5 ± 7.9	24.3 ± 6.1
Bifidobacterium	1.8 ± 1.2	3.2 ± 1.9
Lactobacillus	0.8 ± 0.5	1.5 ± 0.8
Escherichia/Shigella	10.2 ± 4.1	6.5 ± 2.8
Ruminococcus	14.6 ± 5.3	11.2 ± 4.2
Enterococcus	3.5 ± 2.1	2.8 ± 1.5

Table 4 presents the serum levels of key inflammatory markers in PD patients and healthy controls. The data clearly indicate a significant increase in inflammatory activity in the PD group; TNF-α (pg/ml): Tumor Necrosis Factor-alpha is a potent pro-inflammatory cytokine. PD patients had significantly higher levels of TNF-α (15.2 ± 4.8 pg/ml) compared to controls (10.5 ± 3.2 pg/ml), with a p-value of 0.001. This elevation suggests a heightened state of systemic inflammation in PD patients; IL-6 (pg/ml): Interleukin-6 is another important pro-inflammatory cytokine involved in immune regulation. Similar to TNF-α, IL-6 levels were also significantly higher in PD patients (8.7 ± 2.9 pg/ml) compared to controls (5.3 ± 1.8 pg/ml), with a p-value of 0.002. This further supports the presence of increased inflammation in PD; CRP (mg/L): C-reactive protein is an acute-phase protein produced by the liver in response to inflammation. The median CRP level was significantly higher in PD patients (4.5 mg/L) compared to controls (2.1 mg/L), with a p-value of <0.001. This marked elevation in CRP further emphasizes the presence of a systemic inflammatory response in PD patients.

Table 4. Serum levels of inflammatory markers in PD patients and healthy controls.

Marker	PD Patients (n=50)	Healthy Controls (n=50)	P-value
TNF-α (pg/ml)	15.2 ± 4.8	10.5 ± 3.2	1
IL-6 (pg/ml)	8.7 ± 2.9	5.3 ± 1.8	2
CRP (mg/L)	4.5 (2.8-6.2)	2.1 (1.5-3.0)	<0.001

*Data are presented as mean ± SD or median (interquartile range).

Table 5 shows the correlations between specific gut microbiota, inflammatory markers, and measures of disease severity in Parkinson's Disease (PD) patients. This analysis helps to understand the complex interplay between these factors; Faecalibacterium: A moderate negative correlation (Spearman's Rho = -0.45, p = 0.003) was found between the abundance of *Faecalibacterium* and TNF-α levels. This suggests that lower levels of *Faecalibacterium* are associated with higher levels of inflammation. This finding is consistent with the known anti-inflammatory properties of *Faecalibacterium*. A negative correlation (Spearman's Rho = -0.38, p = 0.012) was also observed between *Faecalibacterium* and the Hoehn and Yahr stage, a measure of disease severity. This indicates that lower abundance of this beneficial bacteria might be linked to more advanced PD; Escherichia/Shigella: A moderate positive correlation (Spearman's Rho = 0.52, p = 0.001) was found between *Escherichia/Shigella* and IL-6 levels. This suggests that higher abundance of these potentially harmful bacteria is associated with increased inflammation. A positive correlation (Spearman's Rho = 0.41, p = 0.008) was also observed between *Escherichia/Shigella* and the Non-Motor Symptoms Scale score. This indicates that a higher abundance of these bacteria might be related to a greater burden of non-motor symptoms in PD; Prevotella: A weak negative correlation (Spearman's Rho = -0.32, p = 0.035) was found between *Prevotella* and CRP levels. This suggests that lower levels of *Prevotella* might be associated with higher levels of inflammation, although the correlation is not as strong as for *Faecalibacterium*; Akkermansia: A weak negative correlation (Spearman's Rho = -0.28, p = 0.052) was observed between *Akkermansia* and TNF-α, but it was not statistically significant (p > 0.05). This suggests a potential trend towards lower *Akkermansia* abundance being associated with higher inflammation, but further investigation is needed; Ruminococcus: A weak positive correlation (Spearman's Rho = 0.25, p = 0.081) was found between *Ruminococcus* and the Hoehn and Yahr stage, but it was not statistically significant. This suggests a potential trend towards higher *Ruminococcus* abundance being associated with more advanced PD, but further research is needed to confirm this.

Table 5. Correlation analysis of gut microbiota, inflammatory markers, and disease severity in PD patients.

Gut microbiota	**Inflammatory Marker/Disease Severity Measure**	**Spearman's Rho**	**P-value**
Faecalibacterium	TNF-α (pg/ml)	-0.45	0.003
	Hoehn and Yahr Stage	-0.38	0.012
Escherichia/Shigella	IL-6 (pg/ml)	0.52	0.001
	Non-Motor Symptoms Scale Score	0.41	0.008
Prevotella	CRP (mg/L)	-0.32	0.035
Akkermansia	TNF-α (pg/ml)	-0.28	0.052
Ruminococcus	Hoehn and Yahr Stage	0.25	0.081

4. Discussion

This study aimed to explore the intricate connection between the gut microbiome, inflammation, and Parkinson's Disease (PD) in a specific population in Jakarta, Indonesia. The primary objective was to characterize the gut microbiome composition of individuals with PD and compare it to that of healthy controls. The study also sought to identify potential early biomarkers associated with gut dysbiosis and inflammation, and to explore the possible therapeutic implications of targeting the gut-brain axis in PD. The study found distinct differences in the gut microbiome composition between PD patients and healthy controls. PD patients exhibited a notable reduction in beneficial bacteria, such as *Faecalibacterium*, *Prevotella*, *Akkermansia*,

Bifidobacterium, and *Lactobacillus*. Conversely, there was an increase in potentially harmful bacteria, particularly those belonging to the *Enterobacteriaceae* family. These findings suggest a state of gut dysbiosis in PD patients, potentially contributing to disease pathogenesis. The study also revealed significantly higher levels of inflammatory markers in the blood of PD patients compared to healthy controls. Specifically, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) were all elevated in the PD group. This finding suggests a heightened state of systemic inflammation in PD patients, which may be linked to the observed gut dysbiosis. Correlation analysis demonstrated significant associations between specific gut bacteria, inflammatory markers, and disease severity in PD patients. Notably, there was a negative correlation between the abundance of *Faecalibacterium* and both TNF-α levels and disease severity as measured by the Hoehn and Yahr scale. Conversely, a positive correlation was found between the abundance of *Enterobacteriaceae* and both IL-6 levels and disease severity. These findings suggest a complex interplay between gut microbiota, inflammation, and the progression of PD. The identification of specific gut bacteria and inflammatory markers associated with PD raises the possibility of using these factors as early biomarkers for the disease. Additionally, the findings support the potential of targeting the gut-brain axis for developing novel therapeutic strategies for PD. This study provides valuable insights into the role of the gut microbiome and inflammation in PD pathogenesis. The findings suggest that gut dysbiosis may be an important contributor to the disease process, potentially through mechanisms such as increased intestinal permeability, altered neurotransmitter production, and systemic inflammation. The identification of specific gut bacteria and inflammatory markers associated with PD may pave the way for the development of early diagnostic tools and targeted therapies.[11,12]

Our study delved into the intricacies of the gut microbiome in Parkinson's Disease (PD) patients from Jakarta, Indonesia, revealing notable alterations compared to healthy individuals. These findings align with a growing body of research demonstrating gut microbiome dysbiosis as a potential hallmark of PD across diverse populations. However, our study holds particular significance as one of the pioneering investigations into the gut microbiome of PD patients in Indonesia, offering valuable insights into the potential role of gut-brain axis dysfunction within this specific population. Among the most striking findings is the significant decrease in *Faecalibacterium prausnitzii* within the gut microbiome of PD patients. This bacterium, a member of the *Clostridium leptum* group, is a prominent commensal inhabitant of the human gut, known for its abundance and crucial role in maintaining intestinal health. *F. prausnitzii* is an obligate anaerobe, thriving in the oxygen-deprived environment of the human gut. It plays a pivotal role in the fermentation of dietary fibers, producing butyrate as a primary byproduct. Butyrate, a short-chain fatty acid (SCFA), has garnered considerable attention for its multifaceted benefits. It serves as the primary energy source for colonocytes, the cells lining the colon, promoting their health and integrity. Butyrate also exerts anti-inflammatory effects by inhibiting the activation of nuclear factor-kappa B (NF-κB), a key regulator of immune responses and inflammation. Furthermore, butyrate has demonstrated neuroprotective properties, potentially safeguarding brain cells against damage and degeneration. The observed reduction in *F. prausnitzii* may have profound implications for PD pathogenesis. A decrease in this beneficial bacterium could lead to a decline in butyrate production, potentially contributing to a cascade of detrimental effects. Firstly, the reduced butyrate availability may compromise the integrity of the intestinal barrier, leading to increased intestinal permeability, often referred to as "leaky gut." This compromised gut barrier allows bacterial metabolites, such as lipopolysaccharide (LPS), and other toxins to infiltrate the bloodstream, triggering systemic inflammation. This inflammation, in turn, may exacerbate neuroinflammation, a chronic inflammatory state in the brain that is increasingly recognized as a critical factor in neurodegenerative diseases like PD. Secondly, butyrate plays a role in regulating the

production of neurotransmitters, such as dopamine and serotonin, which are essential for motor control, mood regulation, and cognitive function. A decrease in butyrate production may disrupt this delicate balance, potentially contributing to the motor and non-motor symptoms observed in PD. Another noteworthy observation is the increase in *Enterobacteriaceae* within the gut microbiome of PD patients. This family encompasses a diverse group of Gram-negative bacteria, including *Escherichia coli*, *Shigella*, *Salmonella*, and *Klebsiella*, some of which are known to be pathogenic. These bacteria are notable for their production of lipopolysaccharide (LPS), a potent immunostimulatory molecule that can activate microglia, the resident immune cells of the brain. This activation can trigger neuroinflammation, potentially contributing to the neuronal damage and dysfunction characteristic of PD. The elevated levels of serum inflammatory markers observed in our study further corroborate the role of gut-derived inflammation in PD pathogenesis. The increase in pro-inflammatory *Enterobacteriaceae* and the subsequent release of LPS may contribute to the systemic inflammation observed in PD patients, potentially exacerbating the disease process. It is crucial to consider the interplay between the depletion of *F. prausnitzii* and the increase in *Enterobacteriaceae*. The decrease in *F. prausnitzii* and the subsequent reduction in butyrate production may create a more favorable environment for the proliferation of *Enterobacteriaceae*. Butyrate has been shown to inhibit the growth of certain Gram-negative bacteria, including some members of the *Enterobacteriaceae* family. Thus, the decrease in butyrate may contribute to the overgrowth of these potentially harmful bacteria, further exacerbating gut dysbiosis and inflammation. These findings underscore the complex interplay between the gut microbiome and the central nervous system in PD. The alterations observed in the gut microbiome, particularly the depletion of *F. prausnitzii* and the increase in *Enterobacteriaceae*, may contribute to a cascade of events leading to inflammation and neurodegeneration. This highlights the potential of targeting the gut microbiome as a novel therapeutic strategy for PD.[13-15]

Our investigation into the gut microbiome's role in Parkinson's Disease (PD) extended beyond merely identifying differences in bacterial composition between PD patients and healthy controls. We sought to delve deeper, exploring the potential connections between specific gut bacteria, inflammatory markers, and the severity of PD in our cohort from Jakarta, Indonesia. To achieve this, we employed correlation analysis, a statistical method that allows us to assess the relationships between different variables. This analysis unveiled intriguing associations, providing further evidence for the intricate interplay between the gut microbiome, inflammation, and PD progression. One of the most striking findings from our correlation analysis was the significant negative correlation between the abundance of *Faecalibacterium* and disease severity in PD patients. This correlation indicates that as the abundance of *Faecalibacterium* decreases in the gut, the severity of PD symptoms tends to increase. This observation aligns with the previously discussed beneficial roles of *Faecalibacterium*, particularly its production of butyrate. Butyrate, a short-chain fatty acid (SCFA) produced by *Faecalibacterium* through the fermentation of dietary fibers, is a crucial player in maintaining gut health and overall well-being. It serves as the primary energy source for colonocytes, the cells lining the colon, promoting their health and integrity. Butyrate also exerts potent anti-inflammatory effects by inhibiting the activation of nuclear factor-kappa B (NF-κB), a key regulator of immune responses and inflammation. Furthermore, butyrate has demonstrated neuroprotective properties, potentially safeguarding brain cells against damage and degeneration. The negative correlation between *Faecalibacterium* abundance and disease severity suggests that this bacterium may play a protective role in PD. A decrease in *Faecalibacterium* and its associated butyrate production could have several detrimental consequences. Firstly, the reduced butyrate availability may compromise the integrity of the intestinal barrier, leading to increased intestinal permeability, often referred to as "leaky gut." This compromised gut barrier allows bacterial metabolites, such as lipopolysaccharide (LPS), and other toxins to

infiltrate the bloodstream, triggering systemic inflammation. This inflammation, in turn, may exacerbate neuroinflammation, a chronic inflammatory state in the brain that is increasingly recognized as a critical factor in neurodegenerative diseases like PD. Secondly, butyrate plays a role in regulating the production of neurotransmitters, such as dopamine and serotonin, which are essential for motor control, mood regulation, and cognitive function. A decrease in butyrate production may disrupt this delicate balance, potentially contributing to the motor and non-motor symptoms observed in PD. In contrast to the protective association observed with *Faecalibacterium*, our analysis revealed a significant positive correlation between the abundance of *Enterobacteriaceae* and disease severity in PD patients. This correlation indicates that as the abundance of *Enterobacteriaceae* increases in the gut, the severity of PD symptoms also tends to increase. This observation is consistent with the pro-inflammatory nature of *Enterobacteriaceae*, particularly their production of lipopolysaccharide (LPS). *Enterobacteriaceae* are a large family of Gram-negative bacteria that inhabit the human gut. While some members of this family are commensal and play beneficial roles in digestion and nutrient absorption, others are opportunistic pathogens that can cause infections and contribute to inflammation. These bacteria are characterized by their outer membrane, which contains LPS, a potent immunostimulatory molecule. LPS can activate microglia, the resident immune cells of the brain, triggering neuroinflammation. This neuroinflammation can contribute to neuronal damage and dysfunction, potentially exacerbating PD progression. The positive correlation between *Enterobacteriaceae* abundance and disease severity suggests that these bacteria may contribute to disease progression through their pro-inflammatory effects. The findings from our correlation analysis provide further support for the involvement of the gut microbiome in PD pathogenesis. The observed correlations suggest that specific gut bacteria may be linked to inflammation and disease severity in PD. This information could be valuable for developing targeted interventions aimed at modulating the gut microbiome to potentially improve outcomes in PD patients. The negative correlation between *Faecalibacterium* abundance and disease severity suggests that strategies to increase the abundance of this beneficial bacterium may be beneficial in PD. This could potentially be achieved through dietary modifications, prebiotics (non-digestible food ingredients that promote the growth of beneficial bacteria), or probiotics (live microorganisms that confer health benefits when administered in adequate amounts). Conversely, the positive correlation between *Enterobacteriaceae* abundance and disease severity suggests that strategies to decrease the abundance of these potentially harmful bacteria may be warranted. This could potentially be achieved through dietary modifications, antibiotics, or other approaches to modulate the gut microbiome.[16-18]

The mechanisms by which gut dysbiosis may contribute to the pathogenesis of Parkinson's Disease (PD) are complex and multifaceted, involving a complex interplay of factors and pathways. While the exact mechanisms are still being elucidated, research suggests that gut dysbiosis can trigger a cascade of events that ultimately lead to neuroinflammation and neurodegeneration, the hallmarks of PD. One of the key mechanisms linking gut dysbiosis to PD is the disruption of the intestinal barrier, leading to increased intestinal permeability, often referred to as "leaky gut." The intestinal barrier is a complex structure that plays a crucial role in maintaining gut health and overall well-being. It consists of a single layer of epithelial cells lining the gut, held together by tight junctions, which act as gatekeepers, regulating the passage of substances between the gut and the bloodstream. In a healthy gut, the intestinal barrier selectively allows the absorption of nutrients while preventing the entry of harmful substances, such as bacterial metabolites and toxins. However, gut dysbiosis can disrupt this delicate balance. Alterations in the gut microbiome, particularly the depletion of beneficial bacteria like *Faecalibacterium prausnitzii* and the increase in pro-inflammatory bacteria like *Enterobacteriaceae*, can compromise the integrity of the intestinal barrier. The depletion of *F. prausnitzii* may contribute to leaky gut due to the reduced

production of butyrate, a short-chain fatty acid (SCFA) that serves as the primary energy source for colonocytes, the cells lining the colon. Butyrate promotes the health and integrity of the intestinal barrier, and its deficiency can weaken the barrier's function. The increase in pro-inflammatory bacteria like *Enterobacteriaceae* can also contribute to leaky gut. These bacteria produce lipopolysaccharide (LPS), a potent immunostimulatory molecule that can trigger inflammation and damage the intestinal barrier. The compromised gut barrier allows bacterial metabolites, such as LPS, and other toxins to infiltrate the bloodstream, triggering systemic inflammation. This systemic inflammation is thought to contribute to neuroinflammation, a chronic inflammatory state in the brain that is increasingly recognized as a critical factor in neurodegenerative diseases like PD. Gut microbiome dysbiosis can also affect the production of neurotransmitters, such as dopamine and serotonin, which are essential for motor control, mood regulation, and cognitive function. The gut-brain axis, a bidirectional communication system between the gut and the brain, involves a complex interplay of neural, endocrine, and immune pathways. The gut microbiome plays a crucial role in this communication, influencing various brain functions. Studies have shown that certain gut bacteria can produce dopamine precursors, and alterations in their abundance may influence dopamine levels in the brain. Dopamine is a neurotransmitter that plays a key role in motor control, motivation, and reward. Its deficiency is a hallmark of PD, leading to the characteristic motor symptoms of the disease. Gut dysbiosis can disrupt the production and regulation of dopamine, potentially contributing to the motor symptoms of PD. For example, the depletion of *F. prausnitzii* may reduce the production of butyrate, which has been shown to influence dopamine synthesis and signaling in the brain. In addition to increased intestinal permeability and altered neurotransmitter production, other potential mechanisms may link gut dysbiosis to PD pathogenesis. Gut bacteria produce a variety of metabolites, some of which can have neuroactive properties. Alterations in the gut microbiome can lead to changes in the production of these metabolites, potentially influencing brain function and contributing to PD. Gut dysbiosis can activate the immune system, leading to the production of pro-inflammatory cytokines that can cross the blood-brain barrier and contribute to neuroinflammation. The vagus nerve, a major component of the gut-brain axis, can transmit signals from the gut to the brain. Gut dysbiosis can alter vagus nerve signaling, potentially influencing brain function and contributing to PD.[19,20]

5. Conclusion

This study investigated the gut microbiome composition, identified potential early biomarkers, and explored the therapeutic potential of targeting the gut-brain axis in PD patients in Jakarta, Indonesia. The study found that PD patients exhibit significant alterations in gut microbiome composition compared to controls, with a decrease in beneficial bacteria (e.g., Faecalibacterium prausnitzii) and an increase in pro-inflammatory bacteria (e.g., Enterobacteriaceae). Elevated levels of serum inflammatory markers were also observed in PD patients. Correlation analysis revealed associations between specific gut microbiota, inflammatory markers, and disease severity. The study provides evidence of gut-brain axis dysfunction in PD patients in Jakarta, Indonesia. Alterations in gut microbiome composition and increased systemic inflammation may serve as potential early biomarkers and therapeutic targets for PD. Further research is needed to explore the causal relationship and develop targeted interventions. The findings of this study have important implications for the understanding and management of PD. The gut microbiome may play a key role in the pathogenesis of PD, and targeting the gut-brain axis may offer novel therapeutic opportunities. This study has some limitations, including the small sample size and the cross-sectional design. Future research should include a larger sample size and a longitudinal design to assess the causal relationship between gut dysbiosis and PD. Despite these limitations, this study provides valuable insights into the role of the gut microbiome in PD pathogenesis. The findings suggest that gut dysbiosis may be an important contributor to the disease

process, and that targeting the gut-brain axis may offer novel therapeutic opportunities.

6. References

1. Grahl MVC, Andrade B da S, Perin APA, Neves GA, Duarte L de S, Uberti AF, et al. Could the urease of the gut bacterium Proteus mirabilis play a role in the altered gut-brain talk associated with Parkinson's disease? Microorganisms. 2023; 11(8).
2. Yang Y, Stewart T, Zhang C, Wang P, Xu Z, Jin J, et al. Erythrocytic α-synuclein and the gut microbiome: Kindling of the gut-brain axis in Parkinson's disease. Mov Disord. 2021; 39(1): 40–52.
3. Soni D, Upadhayay S, Dhureja M, Arthur R, Kumar P. Crosstalk between gut-brain axis: unveiling the mysteries of gut ROS in progression of Parkinson's disease. Inflammopharmacology. 2022; 32(5): 2921–41.
4. Neufeld PM, Nettersheim RA, Matschke V, Vorgerd M, Stahlke S, Theiss C. Unraveling the gut-brain axis: the impact of steroid hormones and nutrition on Parkinson's disease. Neural Regen Res. 2021; 19(10): 2219–28.
5. Ebedes D, Borlongan CV. Going straight for the gut: gut-brain axis pathology and treatment of Parkinson's disease. Neural Regen Res. 2022; 19(10): 2111–2.
6. Munteanu C, Onose G, Rotariu M, Poştaru M, Turnea M, Galaction AI. Role of Microbiota-derived hydrogen sulfide (H2S) in modulating the gut–brain axis: Implications for Alzheimer's and Parkinson's disease pathogenesis. Biomedicines. 2021; 12(12): 2670.
7. Park SJ, Kim KW, Lee EJ. Gut-brain axis and environmental factors in Parkinson's disease: bidirectional link between disease onset and progression. Neural Regen Res. 2022.
8. Gui J, Sun X, Wen S, Liu X, Qin B, Sang M. Resveratrol protects dopaminergic neurons in a mouse model of Parkinson's disease by regulating the gut-brain axis via inhibiting the TLR4 signaling pathway. Nan Fang Yi Ke Da Xue Xue Bao. 2021; 44(2): 270–9.
9. Yan Y, Tennekoon K, Eubanks A, Herekar A, Shimoga D, Ayyala D, et al. Evaluation of bidirectional gut-brain axis and anorectal function in Parkinson's disease with constipation. Neurogastroenterol Motil. 2022; 36(3): e14728.
10. Yu L, Hu X, Xu R, Zhao Y, Xiong L, Ai J, et al. Piperine promotes PI3K/AKT/mTOR-mediated gut-brain autophagy to degrade α-Synuclein in Parkinson's disease rats. J Ethnopharmacol. 2021; 322: 117628.
11. Gao V, Crawford CV, Burré J. The gut-brain axis in Parkinson's disease. Cold Spring Harb Perspect Med. 2022; a041618.
12. Wanyi Z, Jiao Y, Wen H, Bin X, Xuefei W, Lan J, et al. Bidirectional communication of the gut-brain axis: new findings in Parkinson's disease and inflammatory bowel disease. Front Neurol. 2021; 15: 1407241.
13. Riegelman E, Xue KS, Wang J-S, Tang L. Gut-brain axis in focus: Polyphenols, Microbiota, and their influence on α-synuclein in Parkinson's disease. Nutrients. 2021; 16(13): 2041.
14. Cansız D, Ünal İ, Gani Sürmen M, Sürmen S, Sezer Z, Beler M, et al. Gentisic acid exerts neuroprotective effects in neurotoxin-induced Parkinson's disease model in zebrafish: Cross-talk between pathways related with neurodegeneration in the gut-brain axis. Brain Res. 2022; 1836(148952): 148952.
15. Ni Y, Tong Q, Xu M, Gu J, Ye H. Gut Microbiota-induced modulation of the central nervous system function in Parkinson's disease through the gut-brain axis and short-chain fatty acids. Mol Neurobiol. 2021.
16. Techaniyom P, Korsirikoon C, Rungruang T, Pakaprot N, Prombutara P, Mukda S, et al. Cold-pressed perilla seed oil: Investigating its protective influence on the gut-brain axis in mice with rotenone-induced Parkinson's disease. Food Sci Nutr. 2020; 12(9): 6259–83.

17. Candeias E, Pereira-Santos AR, Empadinhas N, Cardoso SM, Esteves ARF. The gut-brain axis in Alzheimer's and Parkinson's diseases: The catalytic role of mitochondria. J Alzheimers Dis. 2021; 100(2): 413–29.
18. Houser MC, Tansey MG. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? NPJ Parkinsons Dis. 2017; 3(1): 3.
19. van Laar T. Introduction: The gut-brain axis in Parkinson's disease. J Parkinsons Dis. 2019; 9(s2): S279.
20. Peter I, Strober W. Immunological features of LRRK2 function and its role in the gut-brain axis governing Parkinson's disease. J Parkinsons Dis. 2023; 13(3): 279–96.