Immune Cytokines IFN-γ, TGF-β, TNF-α, and IL-1β Modulate the Pathophysiological Markers in Idiopathic Parkinson's Disease

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Abstract

Background: Parkinson's disease (PD) is a neurodegenerative aging disease, with idiopathic PD being most common. Gastrointestinal tract disorders (GITD) and microbiota changes may trigger idiopathic PD. Neurotoxins from microbiota can travel from the gut to the brain via the brain-gut axis (BGA), leading to α-syn protein misfolding and dopaminergic neuron death. Methods: The aim of the current study was to investigate the link between PD and GITD by measuring several biochemical and immunological markers in 142 patients. The biochemical markers measured were vitamins B6, B12, and D, calcium, serotonin, ghrelin, dopamine, and α-syn protein. The immunological markers included transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interferon-gamma (IFN-γ). All markers were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Results: PD patients were significantly older (63.76±12.29 years) compared to GITD and control groups (41.00±15.54 and 41.25±18.30 years, respectively). Males predominated in the PD group (74.5%), while females were more common in the GITD and control groups. PD and GITD patients showed significantly lower levels of vitamins and neurotransmitters but higher calcium and α-synuclein compared to controls. Immunological markers were elevated in PD and GITD groups, with significant differences between them (P-value < 0.001). Conclusion: The study concluded that certain biochemical and immunological markers provide strong evidence of the brain-gut axis's involvement in the initiation of idiopathic Parkinson's disease.

Keywords: Brain-Gut axis, Dopamine, Vagus nerve, Microbiota, Parkinson's Disease (PD), Gastrointestinal Disorders (GITD), Alpha-synuclein, Neuroinflammation, Biochemical markers

Significance | This study showed the biochemical and immunological links between Parkinson's Disease and gastrointestinal disorders, highlighting shared pathophysiological markers.

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Introduction

Parkinson's disease (PD) is one of the neurodegenerative diseases that occurs gradually, leading to a decline in patients' movement abilities over time. It primarily affects muscle tone and control, causing symptoms such as changes in facial expressions, palsy, and shaking of one or both hands or legs (tremor), unstable posture, bradykinesia, rigidity, difficulties in walking, speech stuttering, amnesia, anosmia, dysphagia, and constipation. These symptoms are mainly due to the death of neurons in the dopaminergic region of the midbrain, specifically in the substantia nigra (SN)（Bloem et al., 2021）.

Research is ongoing to uncover more factors related to PD, including genetic predisposition, exposure to toxins, smoking, pesticides, environmental changes, alterations in gut bacterial communities, certain dairy products, oxidative stress, repeated neuroinflammations, immune system weaknesses, and even head

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trauma. PD has several types, including vascular PD caused by restricted brain blood flow or stroke, drug-induced PD, and idiopathic PD (Pang et al., 2019).

Alpha-synuclein (α-syn) is a native neurotransmitter protein with a unique helical form, kept from misfolding and aggregating by chaperone molecules. Disruptions in this protective system lead to the misfolding and toxic accumulation of α-syn, forming Lewy bodies (LB) and Lewy neurites (LN), which cause the death of neurons in the SN (Meade et al., 2019).

The changing microenvironment in the gut, disruption of internal mucosa and epithelial cell junctions, repeated inflammation of the gastrointestinal tract, and disturbances in digestion mechanisms are significant contributors to PD pathogenesis (W. Wang et al., 2022). Functional links between the brain and gut in both health and disease include vitamins like B6, B12, D (Sokolovska et al., 2022), calcium (Alam et al., 2022), neurotransmitters such as serotonin and dopamine (Nobis et al., 2023), and hormones like ghrelin, which may interact with gut function and contribute to PD initiation (Bayliss & Andrews, 2013). Immune factors also play a crucial role in neuroinflammation, with key players including transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ (IFN-γ) (Perez et al., 2016).

Several gastrointestinal tract disorders (GITDs) such as irritable bowel syndrome (IBS), Helicobacter pylori infection, gastritis, Crohn's disease, and celiac disease may be hidden contributors to idiopathic PD (Hassan et al., 2010; Hazaa & Lami, 2018; Jamil et al., 2017). This study aims to investigate the relationship between PD initiation and some GITDs by measuring biochemical markers like vitamins (B6, B12, D), calcium, neurotransmitters (serotonin, dopamine), ghrelin hormone, and alpha-synuclein protein, in addition to immunological markers like TGF-β, TNF-α, IL-1β, and IFN-γ.

Materials and Methods

Study design:

The current study included 142 (66 females and 76 males) participants, they were attended on hospitals with age range between (20-70 year), they were divided according to cases into 51 PD ,51 GIT patients and 40 healthy or control. The patients and healthy persons were attended to Baghdad hospital in medical city and Al-Yarmouk teaching hospital (Neuroscience and GIT and liver consultations) during the period (March-2022 to September-2022). The patients with PD checked and ensured examination by specialist neuroscientist physicians by serial protocols related with the disease, the patients with GIT disorders detected by endoscopy way (oesophagus-gastro-duodenal scope OGD and colonoscope), in addition to that; health people undergoing theses checked protocols also.

The study design divided the samples in to three sub categories according to age in to three categories (< 30,30-60,> 60 years) and according to duration of disease in to three categories (< 5 ,5-10,>10 years). Peripheral blood was drawing by venipuncture from all the participants. The blood samples were centrifugated to separated serum, with (5000 rpm within 5 min) and then stored in -20C°.

ELISA

The technique that used for tests was enzyme likening immunoassay (ELISA) by direct sandwich antibody antigen combination with used special enzyme-substrate with detection coloration changing in microstrip well, to find concentration of the sample's markers (B6, B12, D) calcium, serotonin, ghrelin, dopamine, α-syn, TGf-1β, TNF-α, IL-1β and IFN-γ) by used special polynomial equation according to Sunlung © and Bioassay Technology laboratory © procedures and software

Statistical analysis:

Analysis of data was carried out using the available statistical package of SPSS-26 (Statistical Packages for Social Sciences- version 26). Data were presented in simple measures of frequency, percentage, mean and standard deviation and ANOVA way.

Results

Demographic characteristics of study groups

The demographic characteristics illustrated in Table 1 revealed that PD patients were the oldest among the three groups, with a mean age of 63.76±12.29 years, compared to the GITD and control groups, which had mean ages of 41.00±15.54 and 41.25±18.30 years, respectively. The differences were highly significant (p-value < 0.001).

The distribution of the study groups according to age subgroups showed that most PD patients (72.5%) were over 60 years old. In contrast, the majority of GITD and control patients were aged 30-60 years, with 56.9% and 40%, respectively. These differences were also highly significant (p-value < 0.001).

Regarding gender distribution, males were more prevalent in the PD group (74.5% vs. 25.5% females). In contrast, females were more prevalent in the GITD group (54.9% vs. 45.1% males) and the control group (62.5% vs. 37.5% males). These differences were highly significant (p-value < 0.001).

The distribution of patients according to disease duration showed that most PD and GITD patients had a disease duration of less than 5 years (70.6% and 72.5%, respectively), with these differences also being highly significant (p-value < 0.001).

Gastrointestinal disorders subgroups

Table 2 illustrates the subgroups of GIT disorders. The total number of patients with GIT inflammatory and immune disorders was 51, subdivided into eight classes based on the nature of the disease, grade of mucosal erosion, appearance of internal cavities in the GIT,

Table 1. Demographic characteristics of study groups

Demographic Characteristic		Groups			
		Parkinson disease	Gastrointestinal tract disorders	Control	p-value
No. (%)		51 (35.9%)	51(35.9%)	40(28.2%)	0.4 NS
Age (Year) (mean ±S.D.)		63.76±12.29	41.00±15.54	41.25±18.30	<0.001***
Age categories	<30	2(3.9%) [c]	14(27.5%) [b]	15(37.5%) [a]	<0.001***
(Year)	30-60	12(23.5%) [b]	29(56.9%) [a]	16(40.0%) [a]	
	>60	37(72.5%) [a]	8(15.7%) [c]	9(22.5%) [b]	
Gender	Female	13(25.5%) [b]	28(54.9%) [a]	25(62.5%) [a]	<0.001***
	Male	38(74.5%) [a]	23(45.1%) [b]	15(37.5%) [b]	
Duration of Disease (Year)	<5	36(70.6%) [a]	37(72.5%) [a]	0(0%)	<0.001***
	5-10	12(23.5%) [b]	11(21.6%) [b]	0(0%)	
	>10	3(5.9%) [c]	3(5.9%) [c]	0(0%)	

P-value= Probability value, ***= Highly significant (<0.001), NS= non-significant. Different small letters refer to significant within -groups comparisons, similar small letters refer to non-significant differences within-group comparison

Table 2. Gastrointestinal tract disorders subclinical groups.

Group	Subclinical group	No.	%
GITD	*Heliobacter Pylori* Gastritis	11	21.6
	Gastritis (General)	9	17.7
	Heliobacter Pylori with IBS	8	15.7
	Duodenal ulcer	7	13.8
	Ulcerative colitis	4	7.8
	Celiac	2	3.9
	Crohn's	2	3.9
	Duodenales	2	3.9
	Gastric tumours	2	3.9
	Gastroesophageal reflux disease (GERD)	2	3.9
	IBS only	2	3.9
Total		51	100%

Table 3. Levels of biochemical markers (Vitamins and Calcium) among the study groups

Group		Vitamin B6 (ng/ml)	Vitamin B12 (pg./ml)	Vitamin D (ng/ml)	Calcium (mg/dl)
		Mean ±S. D			
Parkinson disease		11.2±4.4	4.2±1.5	12.6±5.5	10.1±1.2
GITD		10.0±4.2	3.8±1.7	14.1±7.3	8.0±1.3
Control		32.9±9.1	14.5±4.6	43.5±13.2	9.5±0.6
p-value	*Pa*	<0.001***	<0.001***	<0.001***	<0.001***
	Pb	<0.001***	<0.001***	<0.001***	<0.001***
	Pc	0.1 NS	0.2 NS	0.2 NS	<0.001***

P-value= Probability value, ***= Highly significant (<0.001), NS= non-significant. Pa comparison between Parkinson and control, Pb comparison between GITD and control, Pc comparison between PD and GITD.

Table 4. Levels of neurotransmitters, ghrelin hormone, and alpha-synuclein protein among the study groups

Group		Serotonin (ng/ml)	Dopamine (ng/ml)	Ghrelin (mg/ml)	Alpha-Synuclein (pg/ml)
		Mean ±S. D			
Parkinson disease		28.9±12.9	28.1±11.6	2.8±1.5	151.0±36.1
GITD		34.3±14.7	53.2±18.6	2.4±1.5	96.2±36.5
Control		117.7±21.5	109.6±26.5	8.7±2.1	53.6±18.8
p-value	*Pa*	<0.001***	<0.001***	<0.001***	<0.001***
	Pb	<0.001***	<0.001***	<0.001***	<0.001***
	Pc	0.12 NS	<0.001***	0.55 NS	<0.001***

P-value= Probability value, ***= Highly significant (<0.001), NS= non-significant. Pa comparison between Parkinson and control, Pb comparison between GITD and control, Pc comparison between PD and GITD.

Table 5. Levels of immunological markers among the study groups

Group		TGF (pg/ml)	TNF (pg/ml)	IL-1β (pg/ml)	IFNϒ (pg/ml)
		Mean ±S. D			
Parkinson diseases		152.2±48.2	155.5±43.7	97.4±23.4	69.3±16.0
GITD		241.6±76.4	196.1±73.4	120.3±32.9	73.7±22.9
Control		105.4±22.3	76.2±14.2	58.4±17.0	37.3±10.2
p-value	*Pa*	<0.001***	<0.001***	<0.001***	<0.001***
	Pb	<0.001***	<0.001***	<0.001***	<0.001***
	Pc	<0.001***	<0.001***	<0.001***	0.26 NS

P-value= Probability value, ***= Highly significant (<0.001), NS= non-significant. Pa comparison between Parkinson and control, Pb comparison between GITD and control, Pc comparison between PD and GITD.

and significant examination findings such as polyps, ulcers, or erythema.

The most common disorder, identified through endoscopic examination and according to gastroenterologists, was infection with Helicobacter pylori (21.6%). Most cases were gastritis associated with this bacterium, followed by ordinary gastritis resulting from indigestion, poisoning, or immune reactions (17.7%). Additionally, there were cases with both Helicobacter pylori infection and irritable bowel syndrome (IBS) (15.7%).

In the middle of the table, there were cases of duodenal ulcer and ulcerative colitis (UC), accounting for 13.8% and 7.8% respectively. Other conditions were less common, each representing 3.9% of the total cases.

Biochemical Markers (Vitamins and Calcium)

Table 3 shows the levels of biochemical markers (vitamins and calcium) among the study groups. A highly significant ($P<0.001$) decrease was found in the levels of vitamins B6, B12, and D in both PD (11.2±4.4 ng/ml, 4.2±1.5 ng/ml, and 12.6±5.5 ng/ml, respectively) and GITD (10.0±4.2 ng/ml, 3.8±1.7 ng/ml, and 14.1±7.3 ng/ml, respectively) groups compared to the control group (32.9±9.1 ng/ml, 14.5±4.6 ng/ml, and 43.5±3.2 ng/ml, respectively). However, no significant differences ($P>0.05$) were recorded in the levels of these vitamins between the PD and GITD groups.

Calcium levels were significantly higher ($P<0.001$) in the PD group (10.1±1.2 mg/dl) and significantly lower ($P<0.001$) in the GITD group (8.0±1.3 mg/dl) compared to the control group (9.5±0.6 mg/dl). Additionally, a highly significant increase ($P<0.001$) in calcium levels was found in the PD group compared to the GITD group.

Neurotransmitters, ghrelin hormone and alpha-synuclein protein

Table 4 shows the levels of neurotransmitters (serotonin and dopamine), ghrelin hormone, and alpha-synuclein. The levels of serotonin, dopamine, and ghrelin were lower in PD (28.9±12.9 ng/ml, 28.1±11.6 ng/ml, and 2.8±1.5 ng/ml, respectively) and in GITD (34.3±14.7 ng/ml, 53.2±18.6 ng/ml, and 2.4±1.5 ng/ml, respectively) compared to the control group (117.7±21.5 ng/ml, 109.6±26.5 ng/ml, and 8.7±2.1 ng/ml, respectively). There were no significant differences between the PD and GITD groups regarding serotonin and ghrelin levels, while the dopamine level was lower in PD compared to GITD.

Levels of alpha-synuclein were higher in both PD and GITD groups (151.0±36.1 pg/ml and 96.2±36.5 pg/ml, respectively) than in the control group (53.6±18.8 pg/ml). Additionally, the level of alpha-synuclein was higher in PD compared to GITD. Statistically, all these results were highly significant among the three study groups (p-value < 0.001).

Immunological markers

Table 5 is notable for illustrating immune markers such as TGF-β, TNF-α, IL-1β, and IFN-γ. An increase in the levels of these markers was observed in the PD group (152.2±48.2, 155.5±43.7, 97.4±23.4, and 69.3±16.0 pg/ml, respectively) and the GITD group (241.6±76.4, 196.1±73.4, 120.3±32.9, and 73.7±22.9 pg/ml, respectively) compared to the control group (105.4±22.3, 76.2±14.2, 58.4±17.0, and 37.3±10.2 pg/ml, respectively).

Additionally, the levels of these markers were higher in the GITD group than in the PD group, except for IFN-γ, which showed no significant difference between the two groups. Statistically, all these findings demonstrated highly significant differences with a p-value of <0.001.

Discussion

Despite numerous articles, idiopathic Parkinson's disease (PD) remains a subject of ongoing research due to various ambiguous factors and its diverse effects on physiological functions (DeMaagd & Philip, 2015). The current study's demographic characteristics present several distinctive points compared to other research. The majority of PD patients were elderly, typically in their sixth decade or older, while participants with gastrointestinal tract disorders (GITD) and controls were predominantly in middle age (30-60 years). Similar to findings by Virameteekul et al. (2021), most PD cases in this study began in the middle and later stages of the fifth decade, initially asymptomatic and often diagnosed late when symptoms became evident and the disease had progressed.

Although elderly individuals are more susceptible to various gastrointestinal disorders due to factors like weakened immunity and mucosal layer integrity (Dumic et al., 2019), our study contradicts Dumic et al.'s observations regarding the age of GITD patients. Instead, our results indicate that most GITD participants were in middle age. This finding could be attributed to factors such as consuming spoiled food, smoking, alcohol consumption, exposure to toxins or drugs, recurrent inflammatory reactions, or psychological factors, as concluded by Liu et al. (2023).

While Cerri et al. (2019) suggested a higher PD risk in men compared to women, our study aligns with articles indicating that females may be more prone to GIT disorders due to steroid hormone-related factors (Narayanan et al., 2021).

Regarding disease duration, most PD and GITD cases in our study were within periods of less than five years, likely due to prolonged medication use such as levodopa and carbidopa for PD and fasting relief drugs for GITD. Patients often require extended periods to manage their conditions as the diseases progress. Consequently, many patients reduce their medication follow-up and consultations, relying on suitable therapies only in necessary situations (Mizuno et al., 2018).

Within the GITD group, several subclinical disorders may contribute to PD progression via the vagus nerve through the brain-

gut axis, including inflammatory or autoimmune disorders (Brudek, 2019; Zeng et al., 2022).

The current study reported levels of various biomarkers, including vitamins B6, B12, D, calcium, serotonin, dopamine, ghrelin hormone, alpha-synuclein, as well as TGF-β, TNF-α, IL-1β, and IFN-γ. Levels of all vitamins were decreased in both PD and GITD compared to controls due to reduced nutritional intake and drug effects, particularly levodopa, impacting B6 absorption and increasing homocysteine accumulation, leading to aging-related disorders and general weakness (Al-Kuraishy et al., 2023; Shen, 2015; Shihab et al., 2022). Vitamin D levels were also lower in PD patients due to reduced consumption of vitamin-rich foods and supplements and decreased sunlight exposure, especially in the elderly (Barichella et al., 2022). In GIT patients, deficiencies in vitamins B6, B12, and D were observed due to inadequate intake, absorption difficulties, and complications from repeated infectious diseases (Masri et al., 2015).

Calcium levels were increased in PD compared to control and GIT, likely due to its role in cellular functions and homeostasis, leading to oxidative stress and disrupted cellular function, particularly in neurons (Tehrani et al., 2020). Conversely, GITD patients showed lower calcium levels due to various factors such as liver issues, diarrhea, endocrine disorders, and gut microbial imbalances (Greco, 2012; Korytny et al., 2021; Wang et al., 2022). This imbalance may correlate with α-synuclein aggregation and mitochondrial dysfunction (Goodwin & Pountney, 2014; Surmeier et al., 2017).

Ghrelin hormone and neurotransmitter levels were decreased in PD and GITD compared to controls, while α-synuclein levels were significantly increased in PD and GITD. Serotonin levels were also decreased in PD and GITD, impacting physiological activities including mood regulation, sleep cycles, and appetite stimulation (Miquel-Rio et al., 2023; Yohn et al., 2017). Serotonin's influence on digestion and motility mechanisms in the gastrointestinal tract may contribute to GITD development, consistent with previous findings (Guzel & Mirowska-Guzel, 2022).

In this study, dopamine levels were found to be decreased in both PD and GITD patients compared to the control group. Reduced dopamine production in the substantia nigra (SN) contributes to PD symptoms, particularly motor symptoms like tremors and dyskinesia, due to the accumulation of dopamine oxidation byproducts, such as DA quinones (DAQs) and 3,4-dihydroxyphenylacetaldehyde (DOPAL) (Zhou et al., 2023). Dopamine also plays a role in the gastrointestinal (GI) tract, where it modulates gut motility, exocrine secretion, and mucosal blood flow. Changes in the mucosal layer, erosion, or inflammatory reactions can alter dopamine receptor expression and reduce its actions (Yang et al., 2021).

Ghrelin, known as the "hunger hormone," is produced mainly in the stomach, small intestine, and pancreas. It regulates appetite and promotes fat storage, with its levels influenced by dopamine regulation. In PD, ghrelin is associated with dopamine production and regulation, and its levels decrease with dopamine reduction. Ghrelin levels are also reduced in inflammatory conditions and H. pylori infection, as well as in gastrointestinal disorders like irritable bowel syndrome (IBS) (Masule et al., 2022; Vivarelli et al., 2013).

Alpha-synuclein, a crucial neurotransmitter protein, becomes dysfunctional and accumulates in deformed structures. The elevated levels of α-synuclein observed in PD and GIT disorders in this study align with previous findings linking PD to gut microbiota and gastrointestinal disorders through the brain-gut axis (BGA) (Rietdijk et al., 2017; Schaeffer et al., 2020).

All immune markers showed higher levels in GITD and PD patients compared to controls, with levels generally higher in GITD than PD except for IFN-γ. Increased inflammation, especially in the gut and brain, contributes to PD initiation, consistent with findings suggesting PD results from continuous immune activity in the gut (Magnusen et al., 2021). TGF-β, TNF-α, IL-1β, and IFN-γ play roles in inflammation and immune response regulation, with dysregulation implicated in PD pathology (Kashima & Hata, 2018; Yan et al., 2014). Despite the significant elevation of IFN-γ in PD and GITD compared to controls, the differences between PD and GITD were not significant. This discrepancy may be attributed to the differential immune mechanisms activated in the brain and gut by interferon (Badanjak et al., 2021; Yin et al., 2017).

Conclusion

In conclusion, this study highlights the variability in levels of biochemical and immunological markers among the study groups, shedding light on potential predictors for the initiation of idiopathic Parkinson's disease (PD) and the underlying connection between PD and certain gastrointestinal tract disorders (GITD). Several markers, including calcium, dopamine, alpha-synuclein-β, TGF-β, TNF-α, and IL-1β, emerged as significant indicators across the study groups, bolstering the evidence for the PD-GITD linkage. These markers serve as crucial factors in understanding the relationship between PD and GITD, potentially offering insights into shared pathophysiological mechanisms.

Moreover, other markers such as vitamins (B6, B12, D), serotonin, ghrelin hormone, and IFN-γ also contributed to the PD-GITD relationship, albeit to a lesser extent. While their roles may not be as pronounced as the aforementioned markers, their presence still underscores the complex interplay between PD and GITD.

Overall, the findings suggest a multifaceted association between PD and GITD, with various biochemical and immunological markers

serving as potential indicators. Further exploration of these markers and their interrelationships could provide valuable insights into the etiology and progression of PD, as well as inform novel therapeutic strategies targeting both neurological and gastrointestinal components of the disease.

Author contributions

O.A.H.M. conceptualized the study design, collected and analyzed data, interpreted biochemical and immunological markers, and drafted and revised the manuscript. M.Q.A.L. contributed to the study's conceptualization, supervised the research process, critically reviewed and interpreted findings, and revised the manuscript for important intellectual content. G.A.G. assisted in data collection and analysis, interpreted biochemical and immunological markers, and contributed to drafting and revising the manuscript. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work, ensuring appropriate investigation and resolution of questions related to accuracy or integrity.

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Competing financial interests

The authors have no conflict of interest.

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