

# Gut Status in Parkinson's Disease: The GutSPark Protocol †

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**Abstract:** The neuropathological hallmark of Parkinson's disease (PD) is the accumulation of alpha-synuclein (AS) aggregates. The identification of AS aggregates in gut biopsy specimens from people with PD may provide an opportunity to identify PD at a very early stage, prior to symptom onset. Changes in gut microbiota and inflammatory conditions (such as periodontitis) may be linked with PD onset/evolution. This project aims to explore the concept of microbiota–gut–brain axis in PD, studying gut biopsy specimens for AS aggregates, oral and intestinal microbiota, associated digestive disorders and oral health, of both patients with PD and controls.

**Keywords:** Parkinson's disease; alpha-synuclein; microbiota–gut–brain axis

## 1. Introduction

The neuropathological hallmark of Parkinson's disease (PD) is the accumulation of misfolded alpha-synuclein (AS) aggregates, leading to neuronal loss in the *substantia nigra* and to dopamine deficiency in the striatum. Both the identification of AS aggregates in gut biopsy specimens from people with PD (PwP) and the reports that this is already evident in prodromal PD open an window of opportunity to identify AS disorders prior to symptoms onset, including PD [1]. Up-to-date pathophysiological models suggest that, prior to central nervous system involvement, the disease establishes peripherally, most likely in the gut, as a result of an intricate interplay between gut microbiota, the host's immune and neural systems, and changes in the intestinal wall permeability—the concept behind the microbiota–gut–brain axis hypothesis [2]. Several studies have explored the connection between PD and certain inflammatory conditions (such as periodontitis) that were found to be interconnected with PD at different disease stages. A shift in gut microbiota, another key component, may facilitate local AS aggregation and the ascending spreading from

the enteric nervous system to the brain. The GutSPark study, with its multidisciplinary essence, further explores the concept of the microbiota–gut–brain axis in PD.

The study aims to: (1) To evaluate symptoms of gastrointestinal dysfunction in PwP; (2) To identify and describe oral health problems among PwP; (3) To study the gut, oral and nasal microbiota in PwP; (4) To confirm the presence of, and to characterize, alpha–synuclein aggregates in gut biopsy specimens of patients with PD; (5) To study the microbiome in gut biopsy specimens of patients with PD; (6) To correlate the burden of gastrointestinal dysfunction, oral health problems, microbiota dysbiosis and gut alpha–synuclein aggregates with disease duration, severity, motor and nonmotor features, ongoing treatment, depression and quality of life (QoL) in PwP.

## 2. Materials and Methods

This is an observational, non-interventional, case-control pilot study, with an enrollment of 18 months. Sample (50 PwP/50 controls): (1) Cases: convenience sample from consenting PwP under active follow-up (Neurology Department of Hospital Garcia de Orta or Portuguese Association of PwP) suitable for enrollment (willingness to participate; ability to understand, provide informed consent and comply with all the proceedings; adult patients with confirmed PD diagnosis according to the appropriate clinical criteria; clinical indication for endoscopy); (2) Controls: convenience sample from consenting patients undergoing upper/lower gastrointestinal endoscopy in the Gastroenterology Department, whose biopsy samples from normal-appearing mucosa are considered exempt from pathological changes. **Variables:** Demographics; General clinical data; PD-related data (for the cases); Screening for non-motor symptoms of PD; Cognitive screening; Evaluation of QoL; Evaluation of Depression; Digestive symptoms evaluation; and Full-mouth examination. Collection, analysis and processing of the biological samples: samples of oral, nasal swabs, feces and endoscopic biopsies obtained from PwP and controls will be processed in order to characterize the microbiota. Processing and analysis of gut biopsy specimens obtained during endoscopy will be fourfold: (1) Optical microscopy, for histological analysis; (2) Immunohistochemistry, for identification/quantification of gut AS aggregates; (3) Electron microscopy, for ultrastructural analysis; (4) Microbiome study. The project will be conducted in accordance with the Helsinki Declaration and will seek approval by the local Ethics Committee. Data collection and analysis will be conducted in compliance with all ethical principles, including appropriate protection of confidentiality of the participants.

## 3. Results and Discussion

According to the current knowledge in this field, we expect PD patients to have, comparing to controls, more gastrointestinal dysfunction, oral health problems, microbiota dysbiosis, and alpha–synuclein aggregates in gut biopsy specimens. Furthermore, we expect to confirm a change in the balance of gut/oral/nasal microbiota towards a more proinflammatory milieu in PD. Overall, these changes are expected to correlate with several of the features of PD (disease duration, severity, motor and nonmotor symptoms, ongoing treatment, depression and QoL) and might provide clues to novel treatment targets and/or approaches in this clinical setting.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Instituto Universitário Egas Moniz (protocol code 113/2021; date of approval: 8 July 2021).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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