

Evaluation of Micronucleus in Buccal Mucosa Samples as a Potential Biomarker for Early Diagnosis of Parkinson's Disease: A Cross-sectional Study

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ABSTRACT

Introduction: Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease as of 2024, with a global prevalence of 1.51 per 1,000 people in 2023. Micronuclei are small structures formed when chromosome fragments or whole chromosomes are not properly incorporated into the daughter nuclei during cell division. Their presence can signal genomic instability, which may be linked to neurodegenerative diseases such as PD. The buccal micronucleus assay is a cytogenetic test that examines micronuclei in cells from the buccal mucosa, offering insights into genetic damage or chromosomal instability, and it has been explored as a potential diagnostic tool for PD.

Aim: To compare the presence of micronuclei in the buccal mucosa samples between individuals with PD and healthy controls, as a potential non invasive indicator for early PD detection.

Materials and Methods: This cross-sectional study was conducted in the Department of Neurology at NIMHANS Hospital, Bengaluru, Karnataka, India from March 2023 to February 2024. A total of 170 participants, including both individuals with PD and healthy controls, were recruited from the Outpatient

Department (OPD) and PD wards of NIMHANS Hospital. Buccal mucosa samples were collected using a moistened wooden tongue spatula, fixed onto glass slides with biofix spray and stained using the Papanicolaou (PAP) method. Microscopic images of these slides were then analysed quantitatively using an image analyser, focusing on the presence of micronuclei in the buccal epithelial cells.

Results: The mean age of the healthy group was 66 years, while the mean age of the Parkinson's group was 60 years, with an overall mean age of 63.14 years. The gender distribution in the healthy group included 38 males and 47 females, whereas the Parkinson's group consisted of 55 males and 30 females. Among the study participants, the frequency of micronuclei occurrence was higher in Parkinson's patients (69.4%) compared to healthy controls (11.8%).

Conclusion: The occurrence of micronuclei was notably greater in individuals with PD when compared to healthy controls, suggesting a higher rate of genomic instability in PD patients. This observation reinforces the potential of micronucleus frequency as a useful biomarker for PD.

Keywords: Genomic instability, Micronuclei, Neurodegenerative disease, Non invasive indicator

INTRODUCTION

The PD is the second most prevalent neurodegenerative disease as of 2024. It is the fastest-growing neurodegenerative condition in terms of case numbers [1,2]. The disease primarily affects older adults and its prevalence has risen significantly due to increasing life expectancy. Between 1990 and 2016, PD cases grew by 74% [1]. Projections suggest that by 2040, over 12 million people will be living with PD [3]. In PD, dopamine-producing neurons in the substantia nigra degenerate. This degenerative neurological disorder impacts both the central and peripheral nervous systems [4]. The disease has a long latency period, with clinical symptoms typically appearing only after 70-80% of dopaminergic neurons have been lost [5]. By the time symptoms appear, the damage has already occurred, emphasising the significance of early detection. Timely intervention could enable neuroprotective treatments [6].

Despite advancements in understanding PD's pathophysiology, early diagnosis remains challenging. Current diagnostic methods primarily rely on clinical symptoms, which manifest only after substantial neuronal damage. This limitation underscores the urgent need for accessible and non invasive biomarkers. Ideal biomarkers for PD should exhibit reproducibility, feasibility, affordability, sensitivity and specificity [7]. DNA damage has emerged as a potential biomarker, offering insights into both diagnostic applications and the underlying pathophysiological mechanisms of neurodegenerative disorders [8].

In neurological diseases like Alzheimer's Disease (AD), DNA damage has shown strong associations [9].

Oral mucosal cells, which originate from ectodermal tissue, may exhibit disease-specific traits similar to neurons, as both derive from the same embryonic source, which also forms the central nervous system [10]. There is a lack of research specifically linking the frequency of buccal mucosal micronuclei to PD in Southern India. This study aimed to evaluate the potential association between micronucleus frequency and PD using exfoliated buccal mucosal cells.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Neurology at NIMHANS Hospital, Bengaluru, Karnataka, India from March 2023 to February 2024. The study was approved by the ethical committee of the National Institute of Mental Health and Neuro Sciences, Bengaluru, Karnataka, India (NIMHANS/41st IEC (BS & NS DIV)/2023, 28-06-2023).

Individuals with PD were recruited from the OPD and PD wards of NIMHANS Hospital. Healthy controls were recruited from the Department of Oral and Maxillofacial Pathology and Oral Microbiology at VS Dental College and Hospital. The healthy population was defined as individuals without PD.

Inclusion criteria: The study included PD patients. The diagnosis of PD in the study participants was confirmed through clinical

evaluation based on the UK Parkinson's Disease Society Brain Bank Criteria [11], including the assessment of symptoms such as bradykinesia, tremor and rigidity, supported by neurological examination and, when necessary, imaging studies.

The study also included healthy individuals with no history of neurological or systemic diseases aged above 50 years.

Exclusion criteria:

- The study excluded participants with systemic conditions such as chronic inflammatory diseases, cancer, autoimmune disorders, infections and type 2 diabetes, as these could confound the results.
- Participants currently taking medications or undergoing treatments that affect saliva composition or mucosal health.
- Participants with oral health issues, such as advanced periodontal disease and extensive dental caries, were also excluded from the study.

Sample size: The sample size was estimated using GPower Software v. 3.1.9.2, considering the effect size to be measured (f) at 50%, a power of the study at 95%, and a margin of error of 5%; the total sample size required was 170. Therefore, each group comprised 85 samples. Convenience sampling was used for collecting samples until the desired sample size was achieved.

Sample collection and preparation: Participants were instructed to wash their mouths with water before the clinical examination. A cytospin smear was then collected from the buccal mucosa using a standard wooden tongue spatula moistened with normal saline. The collected scrapings were spread onto a plain glass slide, immediately fixed using Bio-Fix spray, and stained using the rapid PAP technique. The PAP-stained smears were examined under a microscope, and the presence of micronuclei was evaluated as a key parameter.

Image capture and cytomorphometric analysis: A high-resolution CCD camera connected to a research microscope was used to capture images of the smear at 400x magnification. From each slide, 20 cells were selected, and the microscopic images were captured using Image Progress software. Ten well-defined cells with clear staining and without overlap were chosen for cytomorphometric analysis.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 23.0). Descriptive statistics were used as counts and percentages to show the distribution of the presence and absence of micronuclei among different genders and age groups in healthy subjects and Parkinson's patients. A t-test was used for comparison between healthy individuals and PD patients.

RESULTS

A total of 170 subjects were included in the study, comprising 85 healthy controls and 85 patients diagnosed with Parkinson's Disease (PD). The mean age of the total study population was 63.14 years. The mean age of the healthy control group was 66 years, while the mean age of the PD group was 60 years. In the healthy group, there were 38 males and 47 females, while the PD group included 55 males and 30 females.

In PD patients, the presence of micronuclei was significantly higher compared to healthy individuals (p-value=0.0001) [Table/Fig-1]. Among Parkinson's patients, males exhibited a notably higher frequency of micronuclei (47.1%), while females showed a lower occurrence (22.4%) [Table/Fig-2].

Micronucleus frequency in healthy subjects was highest in the 61-70 years age group (5.9%). In Parkinson's patients, the highest frequency was observed in the 50-60 years age group (41.2%) [Table/Fig-3].

Subject group		Micronucleus		Total	p-value
		Present	Absent		
Healthy	Count	10	75	85	0.0001
	% of total	5.90%	44.10%	50.00%	
Parkinson's patients	Count	59	26	85	
	% of total	34.70%	15.30%	50.00%	
Total	Count	69	101	170	
	% of total	40.60%	59.40%	100.00%	

[Table/Fig-1]: Micronucleus status in healthy and Parkinson's groups.

Subject group				Micronucleus		Total
				Present	Absent	
Healthy	Gender	Male	Count	6	32	38
			% of total	7.1%	37.6%	44.7%
		Female	Count	4	43	47
			% of total	4.7%	50.6%	55.3%
	Total	Count	10	75	85	
		% of total	11.8%	88.2%	100.0%	
Parkinson's patients	Gender	Male	Count	40	15	55
			% of total	47.1%	17.6%	64.7%
		Female	Count	19	11	30
			% of total	22.4%	12.9%	35.3%
	Total	Count	59	26	85	
		% of total	69.4%	30.6%	100.0%	

[Table/Fig-2]: Micronucleus status among male and female subjects within healthy and Parkinson's patients.

Subject group				Micronucleus		Total
				Present	Absent	
Healthy	Age group (years)	50-60	Count	1	13	14
			% of total	1.2%	15.3%	16.5%
		61-70	Count	5	44	49
			% of total	5.9%	51.8%	57.6%
		Above 70	Count	4	18	22
			% of total	4.7%	21.2%	25.9%
	Total		Count	10	75	85
			% of total	11.8%	88.2%	100.0%
Parkinson's patients	Age group (years)	50-60	Count	35	18	53
			% of total	41.2%	21.2%	62.4%
		61-70	Count	15	6	21
			% of total	17.6%	7.1%	24.7%
		Above 70	Count	9	2	11
			% of total	10.6%	2.4%	12.9%
	Total		Count	59	26	85
			% of total	69.4%	30.6%	100.0%
Total	Age group (years)	50-60	Count	36	31	67
			% of total	21.2%	18.2%	39.4%
		61-70	Count	20	50	70
			% of total	11.8%	29.4%	41.2%
		Above 70	Count	13	20	33
			% of total	7.6%	11.8%	19.4%
	Total		Count	69	101	170
			% of total	40.6%	59.4%	100.0%

[Table/Fig-3]: Status of micronucleus in healthy subjects between various age groups.

DISCUSSION

Present study evaluated the presence and frequency of micronuclei, which were found to be higher in patients with Parkinson's Disease (PD) compared to healthy individuals.

Migliore L et al., highlight the role of micronuclei as biomarkers for genomic instability, originating from either chromosome breakage or missegregation events. Research in neurodegenerative diseases such as AD and PD has shown an increased frequency of micronuclei, with AD primarily linked to chromosome missegregation and PD to chromosome breakage. In other neurodegenerative and premature ageing disorders such as ataxia telangiectasia, Werner's syndrome, Down's syndrome (DS), and Cockayne's syndrome, micronucleus frequency also increases with ageing in cultured cells. The study suggests that the buccal micronucleus cytome assay could be useful for detecting cellular changes and increased micronucleus frequency, potentially serving as a diagnostic tool to identify individuals at higher risk for AD, DS, and related disorders [12].

Welch G and Tsai LH examined the mechanisms of DNA damage-mediated neurotoxicity in neurodegenerative diseases, highlighting how impaired DNA repair contributes to neuronal degeneration. They noted that DNA breaks and mutations could influence neuronal diversity and also play a role in the development of age-related neurodegenerative diseases. Their work underscores the significance of genomic location and dysfunctional repair proteins in neuronal health. Additionally, they emphasise the role of DNA damage in neuroinflammation, a central feature of neurodegenerative diseases [13].

Migliore L et al., assessed chromosomal and oxidative DNA damage in peripheral blood leukocytes of patients with untreated PD. The results showed significant increases in spontaneous micronuclei, single-strand breaks and oxidised purine bases in PD patients compared to controls. Fluorescence in-situ hybridisation revealed that the micronuclei in PD patients contained acentric fragments. These findings suggest that chromosomal and oxidative DNA damage is present in the lymphocytes of untreated PD patients [14]. In contrast to present study findings, a study involving 425 participants with and without neurodegenerative diseases found no significant differences in DNA damage, including micronuclei, or other cytotoxicity markers (such as binucleated cells, karyolytic cells, and karyorrhectic cells) between patients and healthy controls [15]. The discrepancy between this study and present study results may be attributed to differences in sample size, methodology, or the specific characteristics of the patient population studied.

Limitation(s)

The study's limitations include a small sample size, which may affect the generalisability of the findings, and a cross-sectional design, which limits the ability to establish causality or track changes over time. Confounding factors such as age, lifestyle and environmental exposures were not fully controlled, and the diagnosis was based on clinical evaluation without advanced imaging or genetic confirmation. Additionally, methodological variability in sample collection and

analysis techniques may have affected the consistency of the results, highlighting the need for further research with larger sample sizes and more standardised protocols.

CONCLUSION(S)

The frequency of buccal micronuclei is significantly elevated in individuals with PD compared to healthy controls. This supports the potential use of buccal micronucleus analysis as a non invasive biomarker for the early detection and monitoring of PD. The increased occurrence of micronuclei in PD patients could reflect underlying genomic instability and cellular damage, which are characteristic of neurodegenerative diseases like Parkinson's. Given the accessibility and ease of obtaining buccal cell samples, this biomarker could serve as an important tool in the early diagnosis of PD, enabling timely interventions and improving disease management. However, further studies involving larger and more diverse patient populations are necessary to validate the utility of buccal micronuclei as a reliable diagnostic tool for PD.

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