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"A COMPARATIVE CYTOMORPHOMETRIC ANALYSIS OF THE ORAL MUCOSA BETWEEN HEALTHY INDIVIDUALS AND PARKINSON'S PATIENTS."

Disha K Davis[1] , HannahSugirthabai RajilaRajendran[2]
1 - (Phd) Research Scholar , Chettinad Hospital & research Institute, Chettinad Academy of Research & Education, Chennai, Tamil Nadu, India
Orcid id – 0009-0000-6271-6280
2- M.S, Ph.D, Professor,Department of Anatomy, Chettinad Hospital & research Institute, Chettinad Academy of Research & Education, Chennai, Tamil Nadu, India
Orcid id – 0000-0002-7708-0163

Corresponding author
HannahSugirthabai RajilaRajendran
Professor
Department of Anatomy
Chettinad Hospital & research Institute,
Chettinad Academy of Research & Education,
IT Highway, Kelambakkam
Chennai, Chengalpattu District,
Tamil Nadu,
India- 603103
Email id - drrajianat@gmail.com

Abstract

Background: Parkinson's disease (PD) is the leading neurodegenerative disorder, with recent research focusing on identifying early signs that may precede its onset. This study aims to investigate alterations in the oral mucosa of PD patients using a stereological approach.

Objective: This study sought to assess the buccal mucosa in Parkinson's patients through cytomorphometry. Specifically, it compared cellular perimeter (CP), nuclear perimeter (NP), and the ratio of nuclear diameter to cellular diameter (N/C) between individuals with Parkinson's disease and healthy controls. Buccal smears were collected from 170 individuals aged 50 and older, divided into two groups: 85 with Parkinson's disease and 85 healthy controls. Samples were obtained using a wooden tongue spatula moistened with normal saline, spread on glass slides, fixed with Biofix spray, and stained using the Papanicolaou method. Microscopic images were analysed cytomorphometrically with an image analyser. Data were analysed using SPSS (version 23), employing independent samples t-tests for group comparisons and ANOVA for age group parameter analysis.

Results: A significant difference ($p<0.01$) in N/C ratio and Cp between healthy and Parkinson's patients age noted. There was no significant difference in Np among the study

groups. **Conclusions:** Cytomorphometric analysis showed significant differences in cytoplasmic perimeter and nuclear-cytoplasmic ratios between Parkinson's patients and healthy controls.

Keywords: cellular area, ImageJ, cytology, h and e, pap, nuclear cytoplasmic ratio, nuclear area, Parkinson's disease, buccal smear

Introduction:

Parkinson's disease is a progressive neurodegenerative disorder that primarily affects movement. It is caused by the gradual loss of dopamine-producing neurons in the brain, particularly in a region called the substantia nigra. This depletion leads to characteristic symptoms such as tremors, rigidity, slow movements, and difficulty with balance and coordination. While the exact cause is not fully understood, both genetic predisposition and environmental factors are believed to play roles in its development [1]. The most dangerous aspects of Parkinson's disease include progressive mobility issues leading to an increased risk of falls and injuries, swallowing difficulties that can result in aspiration pneumonia, and cognitive decline ranging from mild impairment to dementia. Additionally, medication side effects like dyskinesia and psychiatric symptoms, along with the potential complications of deep brain stimulation surgery, contribute to the complexity and challenges of managing the disease. Regular medical supervision and a holistic treatment approach are crucial in mitigating these risks and improving quality of life for patients with Parkinson's disease [2].

Parkinson's disease is diagnosed primarily through clinical evaluation of symptoms such as tremor, bradykinesia, and rigidity, along with assessing response to medication like levodopa. Neurological tests and sometimes imaging may be used to confirm the diagnosis and rule out other conditions causing similar symptoms [3]. Recent research has explored oral mucosa cells as potential early indicators of neurological diseases. These cells are derived from ectodermal tissue and are thought to have embryological connections with the central nervous system.

Exfoliative cytology is a non-invasive, cost-effective, and quick procedure that involves the microscopic evaluation of fixed epithelial cells [4]. The study utilized computer-aided cytomorphometry to compare buccal mucosa cytological features between healthy individuals and Parkinson's disease patients, aiming to enhance the diagnostic value of oral exfoliative cytology.

The aim of this study is to conduct a comparative cytomorphometric analysis of the oral mucosa between healthy individuals and Parkinson's disease patients to identify cellular changes associated with the disease. This approach helps in understanding the impact of Parkinson's disease on oral tissues and may reveal early diagnostic markers or inform treatment strategies. By examining differences in cell morphology, the study seeks to enhance our understanding of disease progression and improve patient care.

Materials and Methods

The present study was conducted in the Department of Neurology in NIMHANS hospital, Bangalore over a period of one-year 2023. The study involved a cytomorphometric analysis of the oral mucosa in patients with PD using smears obtained from buccal mucosa. 85 samples from PD patients collected from op and PD ward in Nimhan's hospital and 85 healthy samples collected from V.S Dental college and hospital. The complete procedure was explained to the patients and informed consent was collected from all the study participants

The clearance from the institutional ethical committee was obtained to undertake the study. Detailed information regarding personal details, history of any adverse habit was recorded.

Subjects were asked to rinse the mouth with water. After clinical examination, cytosmear from buccal mucosa using a standard wooden tongue spatula moistened with normal saline was used to obtain scrapings of buccal mucosa. The scrapings were spread on plain glass slide and immediately fixed in bio-fix spray followed by staining with rapid Papanicolaou (PAP) technique. PAP-stained smears were examined, and following parameters were studied:

1. Cytoplasm (Cytoplasmic streaks/ Granularity/ Vacuolation)
2. Micronucleus
3. Cytoplasmic perimeter (CP)
4. Nuclear perimeter (NP)
5. Nuclear: Cytoplasm Ratio (N: C)

A high-resolution CCD camera attached to a research microscope was used to capture the images of the fields at X 400 magnification. From each slide, the microscopic pictures of 20 cells were captured onto a computer, using Image Progress. 10 clearly defined cells with good staining and with no overlap were cytomorphometrically analysed using image J software for CP and NP in pixels. Then, it was calibrated in microns using an equation.

The values CP and NP were entered in excel sheet and average for each case is calculated. The presence of micronucleus and cytoplasmic changes like streaks/ granularity and vacuolation were also assessed.

Statistical Analysis: The data was analysed using SPSS (Statistical Package for Social Sciences version 23) software. Independent sample T test was carried out to calculate between the groups and. Anova is used for analysing the parameters within the different age groups.

Results & Discussion

In this study, we analysed buccal cells from patients with Parkinson's disease, the most common neurodegenerative disorder, and compared them to cells from healthy individuals. We observed differences in cell parameters (CP) and the nuclear/cytoplasmic area ratio between the two groups. Notably, a higher proportion of Parkinson's patients had micronuclei present compared to healthy controls.

Exfoliative cytology has long been employed for the screening and early detection of cancers [5]. The buccal mucosa cells can be easily collected through this affordable, non-invasive method, which is comfortable- for patients. Stereological analysis of these buccal cells offers objective data derived from mathematical and statistical techniques [6].

Exfoliative cytology is a straightforward and non-invasive diagnostic approach that allows for the early detection of lesions. However, its use was constrained by the subjective nature of its analysis and the prevalence of false-negative results. To address these challenges, quantitative methods like image analysis systems were developed. These techniques, including

morphometry, facilitate the examination of differences in cell and nuclear size, shape, and staining intensity [4]. In any cellular modification, the core issue originates at the molecular scale, leading to a series of reactions that impact the overall structure and function of the cell. The genetic activity is evident in the nucleus, while the functional processes are observable in the cytoplasm [7].

Similar to this study several authors have assessed cytomorphometry in buccal mucosa in different conditions. Keles et al and Jajarm HH et al found that there is a significant increase in nuclear volumes and cytoplasmic volumes in the cells of buccal mucosa were markedly higher after kidney transplantation. Considering the heightened risk of cancer in radiation therapy recipients, the authors suggested that the detected alterations might be linked to the cancerous transformation of oral mucosal cells [8]. In the study by Alberti et al. (2003) the researchers found that there was a significant increase in the nuclear-to-cytoplasmic (N/C) ratio in the oral mucosa cells of type II diabetic patients compared to non-diabetic controls. This increase in the N/C ratio is associated with the cellular changes often observed in diabetic patients, reflecting alterations in cell morphology and potentially indicating cellular stress or pathology related to diabetes [9,10]. Karthik KR et al analysed an increase in nuclear volume and the nuclear/cytoplasmic area ratio in oral mucosa cells in diabetic patients in his studies [11]. In their study, Khandelwal and Solomon (2010) identified specific cytomorphological changes in keratinocytes associated with tobacco use and oral squamous cell carcinoma (OSCC). They observed increased eosinophilia and altered texture and granularity in the cytoplasm of affected cells. Notable changes in nuclear features included increased nuclear size and irregular contours. A key finding was the increased nuclear-to-cytoplasmic ratio in cancerous lesions, indicating a larger nucleus relative to the cytoplasm and reflecting heightened nuclear activity in malignant transformation [12]. Buccal mucosa cells come from ectodermal tissue during embryonic development, just like how brain and skin epithelial cells do [13]. Therefore, buccal mucosa cells can be a reflection of alterations in brain tissue.

In the study by de Oliveira et al. (2008), the authors conducted cytologic and cytometric analyses of oral mucosa samples from individuals with Alzheimer's disease. They observed significant alterations in the cytological features of oral mucosal cells in Alzheimer's disease patients. These changes included variations in cell size and morphology, with increased cytoplasmic granularity and irregularities in cell contours. The cytometric analysis revealed changes in the nuclear-to-cytoplasmic ratio, with an increased nuclear size and reduced cytoplasmic volume. This indicated an alteration in cellular structure associated with Alzheimer's disease [14].

In the study by Balan et al., they demonstrated that normal hormonal fluctuations associated with the menstrual cycle cause significant cytomorphometric changes in the oral mucosa, including variations in the NC ratio, nuclear diameter, and cytoplasmic diameter. Specifically, the NC ratio was found to be significantly higher during the ovulatory phase compared to other phases of the cycle. This indicates that hormonal changes influence the proportion of nuclear to cytoplasmic content in oral epithelial cells. There was a significant increase in nuclear diameter during the mid-cycle (ovulation) compared to the follicular and luteal phases. Similarly, the cytoplasmic diameter showed cyclical changes, with variations observed at different stages of the menstrual cycle [15].

Parkinson's disease, marked by neurodegeneration and systemic stress, can lead to alterations

in cellular structures, including changes in cell size and the nuclear/cytoplasmic ratio. These changes may be due to the direct impact of neurodegeneration, chronic inflammation, and disruptions in cellular function and growth. Such modifications are observed not only in neural tissues but also in other areas like the oral mucosa. Analysing these cellular differences helps to understand the broader systemic effects of Parkinson's disease.

Following figures depict the histologic views of exfoliative cell samples.

Figure 1

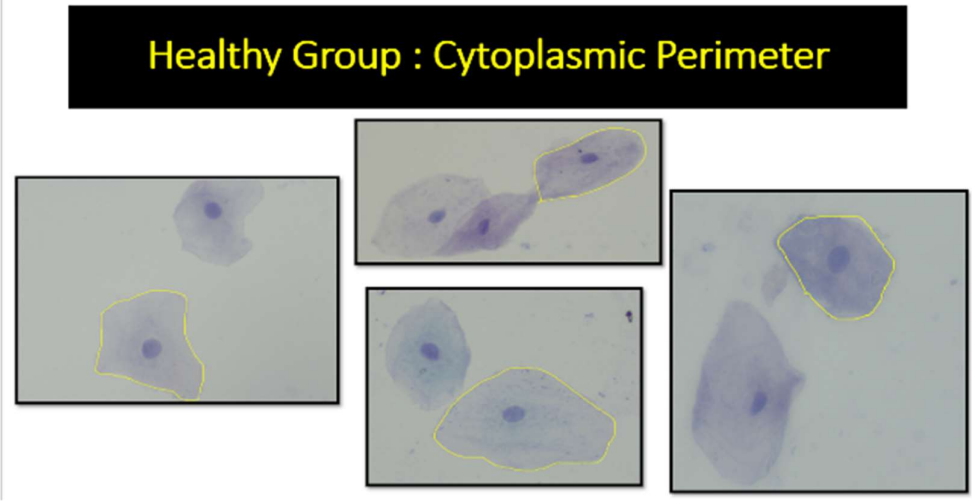


Figure 2

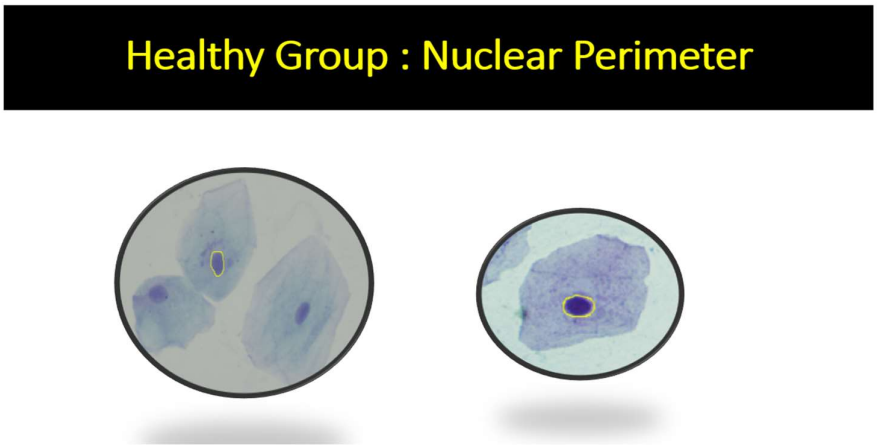


Figure 3

Parkinson's Patients Cytoplasmic Perimeter

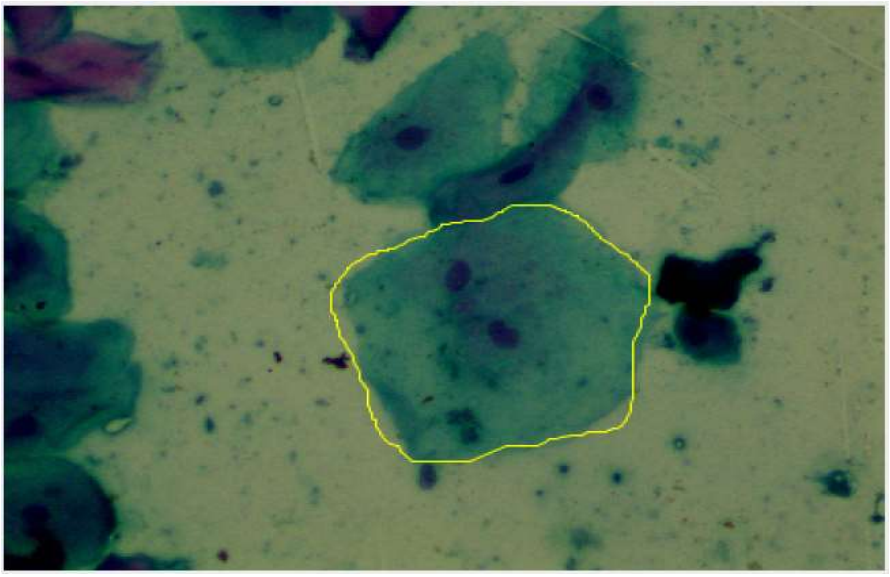
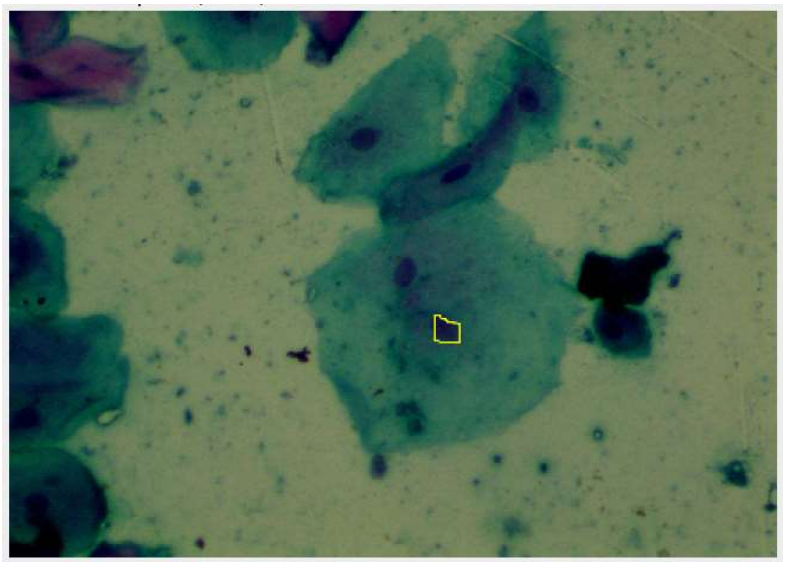


Figure 4

Parkinson's Patients Nucleus Perimeter



Figures 1 through 4 illustrate histologic views of exfoliative cell samples, highlighting distinct cytomorphometric differences between Parkinson's patients and healthy individuals.

Table-1 indicates that the study sample is evenly split between healthy individuals and Parkinson's patients, with a total of 170 participants

Subject group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Healthy	85	50.0	50.0	50.0
	Parkinsons Patients	85	50.0	50.0	100.0
	Total	170	100.0	100.0	

.Table-2 indicates the intergroup comparison

Intergroup Comparisons

Parameters	Group1 (Healthy) Mean ± SD	Group 1I (PD) Mean ± SD	P value
CP	181.74±16.01	200.98±14.95	.000**(<0.01)
NP	35.91 ± 2.83	35.36±2.99	.217(>0.05)
NC_ratio	0.20±0.02	0.17±0.02	.000**(<0.01)

From Intergroup Comparisons based on Health and PD groups on an average more CP is found in Group II (PD) and the difference is highly significant at 1% level of significance. At the same time on an average, more Nuclear- Cytoplasm Ratio is found in Group I (Control) and the difference is highly significant at 1% level of significance but the level of NP on an average equal in both the groups and the difference is not significant

Conclusions

Our study concluded that oral cytological evaluation aids in early diagnosis. Our findings indicate the need for further research to clarify the impact of neurodegenerative diseases on buccal mucosa cells. Additionally, it is important to explore whether these changes can serve as useful clinical predictors of neurodegenerative disease progression.

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