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# OPTICAL COHERENCE TOMOGRAPHY AND DIFFUSION TENSOR IMAGING AS BIOMARKERS OF CLINICAL SEVERITY IN IDIOPATHIC PARKINSON'S DISEASE: A CROSS-SECTIONAL STUDY

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## ABSTRACT

**Background:** Parkinson's Disease (PD) is a progressive neurodegenerative disorder with both motor and non-motor manifestations. Retinal and white matter changes may serve as non-invasive biomarkers for disease severity. The objective is to evaluate retinal nerve fibre layer (RNFL) thickness using optical coherence tomography (OCT) and white matter integrity using diffusion tensor imaging (DTI) in PD patients, and to correlate these findings with clinical severity. **Materials and Methods:** A cross-sectional observational study was conducted on 50 PD patients and 50 age- and gender-matched controls. RNFL thickness was measured using spectral-domain OCT. Fractional anisotropy (FA) and mean diffusivity (MD) of the substantia nigra were assessed via DTI. Clinical severity was evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) and Modified Hoehn and Yahr staging. **Result:** PD patients showed significantly reduced RNFL thickness compared to controls (p=0.001), with negative correlations to disease duration and severity. FA values were significantly lower (p=0.003) and MD values significantly higher (p=0.001) in PD patients. MD correlated positively with disease duration and severity; FA showed a weak negative correlation with severity. **Conclusion:** OCT and DTI metrics correlate with clinical severity in PD and may serve as non-invasive biomarkers for disease monitoring.

## INTRODUCTION

Idiopathic Parkinson's Disease is the prototype of the bradykinetic group of movement disorders. Parkinson's disease (PD) is one of the most common neurodegenerative movement disorders, and with increasing age, its incidence is increasing worldwide. The disease generally begins with motor symptoms of bradykinesia, rigidity, resting tremor, and postural instability. Non-motor symptoms like cognitive impairment, depression, sleep and behavioural problems, and olfactory dysfunction are also seen in patients with PD.

Dopamine depletion is observed to play an important role in the pathogenesis of the disease.[1-5] Such dopaminergic cell loss seen most prominently in the basal ganglia causes the motor symptoms. Dopamine depletion in other parts of the brain and parts of the visual apparatus, including certain layers of the retina, parts of the visual pathway, and layers of the occipital cortex, causes the non-motor deficits.

The dopaminergic cell loss in the retina leads to reduced thickness of the retinal nerve fibre layer (RNFL). This thickness can be measured by optical coherence tomography (OCT). OCT is a noninvasive technology, where-in, cross-sectional images of retinal structures are acquired. This allows to assess the neural fundus integrity and to quantify the structural axonal damage by measuring peripapillary RNFL (PRNFL) thickness. This helps in an indirect estimation of retinal ganglion cell (RGC) layer impairment or directly by estimating macular thickness measurements, since 30%–35% of the retina thickness in the macular area is composed by the RGCs and their fibres.[6]

Diffusion Tensor Imaging is an MRI technique that helps in estimating the changes in white matter integrity, which makes it possible to study the


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structures of cerebral tissue, such as the trajectories in white matter bundles and the orientation of fibres.[7,8]

It is based on the measurement of "the random motion of water molecules in fluid water", particularly suited to neural fibres. Considering the neurodegeneration of both cortical and sub-cortical structures in Parkinson's Disease, it would help to understand the PD pathophysiology from a more global perspective.[9-11]

This study aims to evaluate the OCT and DTI findings in PD patients and try to corroborate the findings with clinical severity.

**Aims and objectives:**

- To find out the thickness of the retinal nerve fibre layer in patients with Idiopathic Parkinson's disease using optical coherence tomography.
- To study the mean diffusivity and fractional anisotropy in patients with Idiopathic Parkinson's disease using diffusion tensor imaging.
- To find out the correlation between the optical coherence tomography and diffusion tensor imaging findings with the clinical severity in patients with Idiopathic Parkinson's disease.

## MATERIALS AND METHODS

**Source of data:** Subjects for the study were recruited from the Department of Neurology, Andhra Medical College, King George Hospital, Visakhapatnam

**Study Design:** Cross-sectional observational study

**Study Period:** April 2019 to December 2020.

**Study Sample:** 50 subjects fulfilling the inclusion criteria for Parkinson's Disease and 50 age and gender matched controls.

**Inclusion criteria:**

- Patients who are over 50 years of age
- Fulfilment of the UKPDS Brain Bank Criteria for the diagnosis of PD.
- Patients who have consented to the study and can undergo required investigations for the study.

**Exclusion Criteria:**

- Glaucoma, intraocular pressure >21 mmHg, history of surgery for glaucoma, or patients on antiglaucoma treatment.
- Media opacity sufficient to preclude optical imaging by OCT
- History of optic neuritis or history of sudden loss of vision in either eye
- Any systemic illness precluding optical imaging by OCT and DTI.
- Parkinson Plus syndromes.
- Secondary Parkinsonism.

**Eligibility criteria for controls:** The controls of patients were age and gender matched. These controls had to satisfy the exclusion criteria mentioned for the PD patients.

Institutional Ethics Committee Approval was obtained prior to the initiation of study.

**Study Procedure:** Patients fulfilling inclusion and exclusion criteria were subjected to detailed case proforma questionnaires followed by neurological examination. The disease severity was evaluated using UPDRS scoring, Modified Hoehn and Yahr staging.

Patients were referred to Government Regional Eye Hospital, Visakhapatnam for undergoing OCT. Visual field testing was done to rule out glaucoma. Fundus examination was done to rule out posterior segment pathologies.

OCT was done for RNFL analysis. Cirrus Spectral Domain-OCT 5000 was used to evaluate RNFL thickness using optic Disc Cube 200 × 200 protocol. Cirrus SD-OCT algorithms identify the optic disc and automatically place a calculation circle with a 3.46-mm diameter evenly around it. Layer-seeking algorithms determine the RNFL inner (anterior) boundary and RNFL outer (posterior) boundary for the entire cube, except the optic disc. For average RNFL thickness, RNFL thickness in the four quadrants (i.e., inferior, superior, nasal, and temporal), and binocular RNFL symmetry, percentages were used. The inner and outer rings were segmented into four quadrants, with radii of 1.5 mm and 3 mm, respectively.

**DTI analysis:** Patients were sent to Department of Radiology, Andhra Medical College for MRI. A 1.5 Tesla MRI machine was used for the DTI analysis. Number of channels in receiver Head Coil were 8, and 21 diffusion directions were used. Region of Interest was applied using the manual method. Fractional Anisotropy and Mean Diffusivity of Substantia Nigra on both sides were calculated. The radiologist and the technician were blinded for the clinical details of the study participant to reduce the bias.

**Study tools:**

- Consent Form
- Case Proforma
- UKPDS Brain Bank Criteria for Idiopathic Parkinson's Disease
- Unified Parkinson's Disease Rating Scale
- Modified Hoehn and Yahr staging for Idiopathic Parkinson's disease.

**Informed consent form:** A self-designed informed consent form, which explained the nature of the study, the contents of which were explained in vernacular language, was read out to the subjects and for those who were willing to participate in the study, signature or left thumbprints in case of illiterates was obtained.

**Case Proforma:** A case proforma to collect personal and socio-demographic details of the subjects has been used. This contains details about identification data like name, age, place of origin, marital status, religion, education, occupation, and income. Details about clinical variables like illness duration, history of the illness, past history, and any comorbid medical illnesses were taken. Neurological examination was done and recorded in the proforma.

**UKPDS Brain Bank Criteria for Idiopathic Parkinson's Disease:** It is the most commonly used criteria for Parkinson's Disease in the United Kingdom. It is three step criteria that clinicians can


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use in their assessments of individuals suspected of having Parkinson's Disease.

**Unified Parkinson's Disease Rating Scale:** UPDRS is used to follow the longitudinal course of Parkinson's Disease. It is one of the most used scales in the clinical study of Parkinson's Disease.

It helps to assess the patient in the following domains.

- Mentation, Behavior and Mood
- Activities of Daily Living
- Motor Examination
- Complications of Therapy
- Modified Hoehn and Yahr Staging
- Schwab and England Activities of Daily Living Scale

**Modified Hoehn and Yahr Staging:** The severity of PD is graded using Modified Hoehn and Yahr Staging as follows:

Stage 1 – Unilateral involvement only

Stage 1.5 - Unilateral and axial involvement

Stage 2 - Bilateral involvement without impairment of balance

Stage 2.5 - Mild bilateral disease with recovery on pull test

Stage 3 - Mild to Moderate bilateral disease with some postural instability, but physically independent

Stage 4 – Severe disability, still able to walk or stand unassisted

Stage 5 – Wheel chair bound or bedridden unless aided

**Statistical analysis:** Analysis of data was done using SPSS-21 version. Descriptive data was presented as frequencies and percentages. Unpaired t-test and ANOVA were applied to find the statistical difference between means. Pearson and Spearman correlation tests were used for measuring the correlation between measures. Data representation was done by appropriate statistical tables and charts.

## RESULTS

The study included 50 subjects fulfilling the inclusion criteria with symptoms of parkinsonism and 50 healthy controls. Cases and controls were matched for age and gender.

[Table 1] shows the distribution of study sample according to age and gender.

**Table 1: Distribution of study sample according to age and gender.**

| Age group | Male | Female | Total |
|---|---|---|---|
| 56-60 | 10 | 06 | 16 |
| 61-65 | 10 | 11 | 21 |
| 66-70 | 04 | 06 | 10 |
| >70 | 00 | 03 | 03 |
| Total | 24 | 26 | 50 |

Duration of the Disease: The mean duration of patients presenting with Parkinson's Disease included in the study was 4.6 + 2.9 years. The duration ranged from 8months to 12 years.

MDS – UPDRS 3 Scores: The mean MDS UPDRS 3 scores of patients presenting with Parkinson's Disease included in the study was 40.04 + 11.6, ranging from 20 to 62.

Distribution of patients according to the Modified Hoehn and Yahr Staging:

Among the patients, 5 (10%) were stage 1, 4 (8%) were stage 1.5, 18 (36%) were stage 2, 3 (6%) were stage 2.5, 12 (24%) were stage 3, and 8 (16%) were stage 4.

**OCT Results:**

**Retinal Nerve Fibre Layer Thickness:**

Retinal Nerve Fibre Layer (RNFL) thickness of both eyes of all the cases and controls were studied in the following four quadrants: Nasal, Temporal, Superior and Inferior.

The average thickness of all the quadrants in each eye and the average global RNFL thickness of all the cases and controls were also studied.

In the right eye, the mean average RNFL thickness of patients with PD was 74.72 + 18.01 µm and that of controls was 85.48 + 9.94 µm. On comparing using the unpaired t-test, it was concluded that the RNFL thickness of PD patients was significantly lower than that of the controls at a p-value of 0.001 which was statistically significant at $p<0.05$.

The data pertaining to RNFL thickness of the right eye quadrants and the average is represented in the below picture.

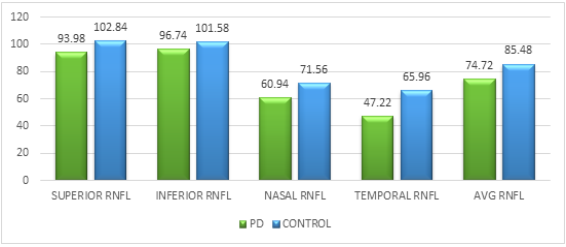


**Figure 1: RNFL thickness of the Right Eye quadrants**

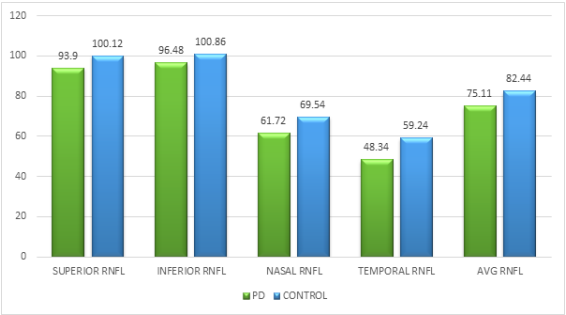


**Figure 2: RNFL thickness of the Left Eye quadrants**

In the left eye, the mean average RNFL thickness of patients with PD was 75.11 + 17.63 µm and that of controls was 82.44 + 11.32 µm. On comparing using the unpaired t-test, it was concluded that the RNFL


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thickness of PD patients was significantly lower than that of the controls at a p-value of 0.015 which was statistically significant at p<0.05.
The data pertaining to RNFL thickness of the left eye quadrants and the average is represented in the below picture.
Average Global RNFL Thickness: The average global RNFL thickness of patients with PD was 74.91 + 17.81 µm and that of controls was 83.96 + 8.08 µm. On comparing using the unpaired t-test, it was concluded that the RNFL thickness of PD patients was significantly lower than that of the controls at a p-value of 0.001 which was statistically significant at p<0.05.

**Table 2: Average Global RNFL Thickness**

| | Group | Mean | Std. Deviation | P VALUE |
|---|---|---|---|---|
| Average Global RNFL µm | PD | 74.9150 | 17.8148 | .001 |
| | Control | 83.9625 | 8.0821 | |

**DTI Results:**

**Fractional anisotropy:**

Fractional anisotropy values were obtained from substantia nigra of the patients and was compared with controls.

The mean Average FA in the patients was 0.463 + 0.051 and in the controls was 0.490 + 0.031. The values were compared using the unpaired t-test and it was found that the mean FA values in the patients were significantly lower compared to the controls with a p-value of 0.003.

**Table 3: Fractional Anisotropy values in patients and controls**

| | Group | Mean | Std. Deviation | P value |
|---|---|---|---|---|
| FA Right | PD | .46308 | .052538 | .002 |
| | Control | .49038 | .031187 | |
| FA Left | PD | .46438 | .051746 | .003 |
| | Control | .49150 | .034026 | |
| AVG FA | PD | .46373 | .051270 | .002 |
| | Control | .49094 | .03139 | |

**Mean diffusivity:** Mean Diffusivity values were obtained from substantia nigra of the patients and was compared with controls.

The mean Average MD in the patients was 0.734 + 0.015 and in the controls was 0.723 + 0.010. The values were compared using the unpaired t-test and it was found that the mean MD values in the patients were significantly higher compared to the controls with a p-value of 0.001.

**Table 4: Mean Diffusivity values in patients and controls**

| | Group | Mean | Std. Deviation | P value |
|---|---|---|---|---|
| MD Right | PD | .73364 | .015775 | .001 |
| | CONTROL | .72228 | .011044 | |
| MD Left | PD | .73524 | .015483 | .001 |
| | CONTROL | .72404 | .010945 | |
| AVG MD | PD | .73444 | .0152845 | .001 |
| | CONTROL | .72316 | .01033 | |

**Correlations:** Global RNFL thickness showed significant correlations with both duration of the disease and clinical severity with correlation coefficients of -0.696 and – 0.467 respectively.

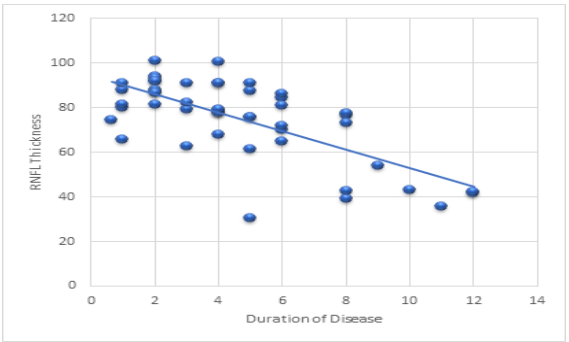


**Figure 3: Correlation between Global Avg. RNFL Thickness and Duration of Disease (r= -0.696 at p<0.001)**

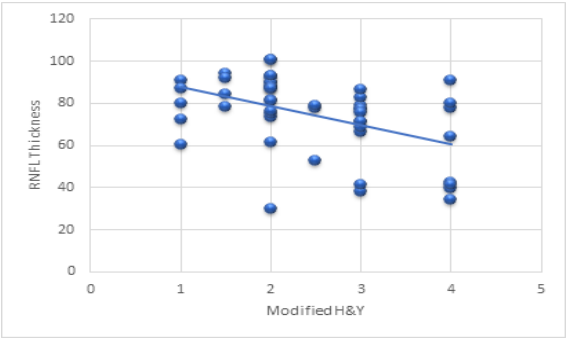


**Figure 4: Correlation between Global Avg. RNFL Thickness and Clinical Severity (r= -0.467 at p<0.001).**

**Fractional Anisotropy vs Duration of Disease:** There was a statistically significant negative correlation between the duration of the disease with a correlation coefficient of -0.583, with a p-value of 0.001


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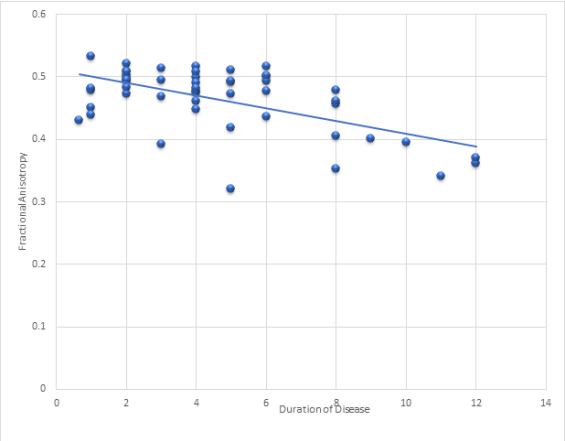


**Figure 5: Correlation between Fractional Anisotropy and Duration of Disease (r= -0.583, with a p-value of 0.001)**

Mean Diffusivity vs Duration of Disease:

Mean Diffusivity values showed statistically significant correlation with both duration of disease and clinical severity with correlation coefficients 0.650 and 0.889 respectively.

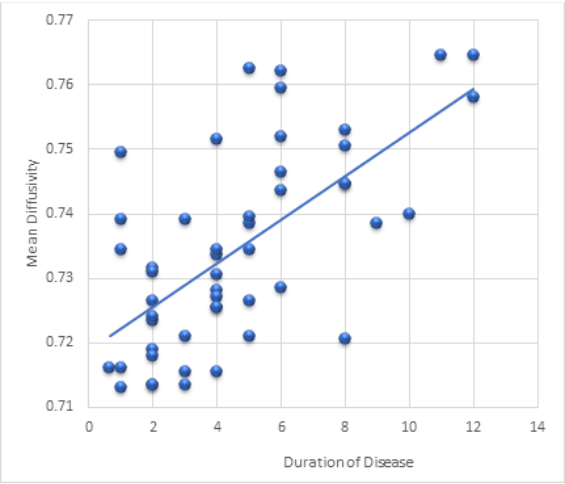


**Figure 6: Correlation between Mean Diffusivity and Duration of Disease (r=0.650, with a p-value of 0.001)**

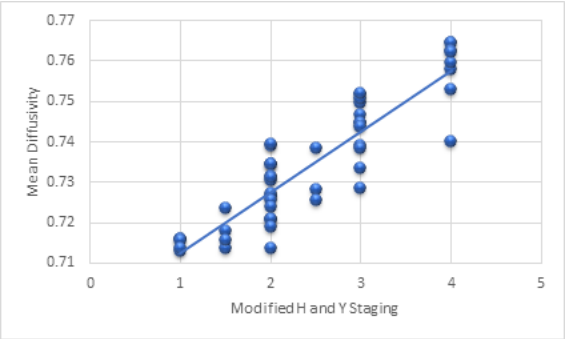


**Figure 7: Correlation between Mean Diffusivity and Clinical Severity (r= 0.889, with a p-value of 0.001.)**

## DISCUSSION

The current study was performed to evaluate the OCT and DTI findings in PD patients and to see if there is any correlation with the clinical severity. It has been assumed that age-related losses in retinal ganglion cells (RGC) thickness affects age-dependent reduction of RNFL by approximately 0.2%/year.[13]

The role of RNFL thickness in PD patients can be justified by the presence of dopaminergic cells in the several parts of retino-optic pathway such as those in the amacrine retinal cells, lateral geniculate body and some parts of the occipital cortex.

As the RNFL is formed by axons of the retinal ganglion cells and since there is degeneration of dopaminergic cells in PD, this may lead to the thinning of RNFL.[14]

Studies have been done to assess the RNFL thickness in PD patients and compare them with healthy controls.

Studies done by Sengupta et al,[14] Rohani et al,[15] concluded that RNFL thickness was significantly reduced in all quadrants in PD patients compared to the controls. Inzelberg et al,[16] observed that the RNFL thickness was reduced in some quadrants like the temporal, inferotemporal and mid-inferior, whereas it was similar in the inferonasal quadrant.

Studies done by Moschos et al,[17] and Sari et al,[18] Statue et al,[19] Sen et al,[20] reported that the RNFL thickness in inferior and temporal quadrants was significantly reduced and in the remaining quadrants, no statistical significance was found.

Polo et al,[21] and La Morgia et al,[22] found that the RNFL thickness was significantly reduced only in the temporal quadrant and in the rest of the quadrants, the thicknesses were comparable between the patients and the controls.

Archibald et al,[23] and Quagliato et al,[24] in their study did not find any statistically significant difference in the RNFL thickness of PD patients when compared to the controls.

There is certain amount of variation among the results of various studies, which could be due to any methodological differences. However, most studies found thinning of RNFL in the PD patients compared to the healthy controls. This study is in line with most of the studies.

With the evidence based on the findings from these studies, OCT has attained importance in the diagnosis of neurodegenerative disorders with ophthalmological involvement such as Parkinson's Disease, Multiple System Atrophy, Multiple Sclerosis etc.[25]

This study found a significant negative correlation between the duration of disease and the RNFL thickness. With the increasing duration of disease, there could be further degeneration of the dopaminergic cells contributing to further reduction of RNFL thickness.

Using ANOVA and post hoc analysis, the mean RNFL thickness across the PD groups was compared with the controls. ANOVA revealed there was a statistically significant difference among the mean values across the groups with a net p-value of 0.001 ($p<0.05$). The mean thickness of the mild PD patients and controls was comparable, but the thickness was significantly different in the moderate ad severe PD


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groups when compared with the controls. This indicates that as the severity of the PD increases, there is a gradual thinning of the retinal nerve fibre layer.

This finding was supported by the significant negative correlation between the severity of disease and the RNFL thickness (p=0.001). This finding implies that the RNFL thickness could be useful as a predictor of the disease progression.

This finding is in line with the findings of an Indian study done by Sengupta et al, who concluded that the clinical severity of disease was negatively correlated to the RNFL thickness. However, certain studies like Elkhatib et al,[26] Matlach et al,[27] did not find any significant correlation between the RNFL thickness and clinical severity. This could have been influenced by parameters like the different medications which determine the progression of the disease.

**Diffusion Tensor Imaging and PD:** Advances in structural and functional imaging have improved the capacity of MRI to detect changes in PD as well as to differentiate between PD and other Parkinsonian syndromes. MRI has provided several candidate biomarkers that have a potential to inform on the disease process. Biomarkers are quantitative characteristics which are used as indicators of biological or pathological states.

In neuroimaging, biomarkers are measures derived from images that reflect the presence of diseases or their severity and that can be used for early diagnosis, prognosis or to monitor responses to therapeutic interventions.

Imaging biomarkers using magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) can describe parameters such as fractional anisotropy (FA) and mean diffusivity (MD). These parameters, when measured in the substantia nigra (SN), have not only shown promising but also varying results. To clarify the potential diagnostic value of substantia nigra DTI in PD, the present study was conducted.

This study found that the mean average FA values in the patients were significantly lower compared to the controls with a p-value of 0.003. The present findings were similar to the study done by Chan et al,[28] where 73 PD patients and 78 controls were taken up for analysis in caudate, globus pallidus, putamen, substantia nigra and thalamus and found out statistical significance in substantia nigra region (p=0.001).

In another study by Guangwei et al,[29] PD subjects overall had significantly reduced FA values in both rostral and caudal segments of the SN compared to control subjects, the FA decrease in the caudal region seemed to be more robust than the rostral region for caudal SN.

Even though many previous studies looked for abnormalities in various brain regions through DTI technique in PD patients, only few studies like Chan et al,[28] Yoshikawa et al,[30] could find differences in FA values in substantia nigra region but couldn't find any changes in other brain regions. Studies conducted by Menke et al,[31] Focke et al,[32] did not show any significance in any regions studied but showed a decreased trend like this study. In an Indian study done by Gopi et al,[33] the mean FA values of substantia nigra regions of PD patients were lower compared to the controls, but statistically not significant. None of the FA values in the studied regions showed statistically significant difference between patients and controls.

**Table 5: Comparison of DTI studies – FA**

| | No.of cases | No.of controls | Region studied | FA values |
|---|---|---|---|---|
| Present study | 50 | 50 | SN | SN ↓ |
| Yoshikawa et al,[30] | 12 | 8 | PMC, SN, BG | SN ↓ |
| Chan et al,[28] | 73 | 78 | GP, PU, SN, TH | SN↓ |
| Menke et al,[31] | 10 | 10 | SN | No variation |
| Focke et al,[32] | 12 | 13 | GP, SN, PU, CA | No variation |

The mean MD values in the patients were significantly higher compared to the controls with a p-value of 0.001.

Various studies have observed that MD values were significantly increased in patients with PD. Study done by Nagae et al,[34] found significant increase in MD values of patients with PD compared to the controls. Studies done by Chan et al,[28] Jiang et al,[35] also had similar findings. However, some studies did not find any significant difference between the MD values of patients and controls. Studies done by Peran et al,[36] Du et al,[37] Zhan et al,[38] found the increase in MD values was not statistically significant.

**Table 6: Comparison of DTI studies – MD**

| | No.of cases | No.of controls | Region studied | MD values |
|---|---|---|---|---|
| Present study | 50 | 50 | SN | SN↑ |
| Nagae et al,[34] | 21 | 20 | SN | SN↑ |
| Chan et al,[28] | 21 | 19 | SN | SN↑ |
| Jiang et al,[35] | 31 | 34 | SN | SN↑ |
| Peran et al,[36] | 30 | 22 | SN | No variation |
| Du et al,[37] | 16 | 16 | SN | No Variation |
| Zhan et al,[38] | 12 | 20 | SN | No Variation |


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There was a significant negative correlation between the duration of the disease and the fractional anisotropy values (p=0.001). However, the clinical severity and FA values had only a weak negative correlation with no statistical significance (p=0.183). This is in contrast to a study done by Prakash et al,[39] there was a significant positive correlation between the clinical severity and FA ($p < 0.03$), observed only at the left rostral SN. There were no significant correlations between the DTI indices with age or disease duration.

In a meta-analysis by Zhang et al,[40] many studies (Nagae et al,[34] Peran et al,[36] Du et al,[37] Zhan et al,[38]) have reported significant correlations between decreased FA in the SN and increased severity of the motor symptoms, which are assessed by the motor exams (part-III) of the Unified Parkinson's Disease Rating Scale (UPDRS) or H&Y scales. However, some other studies (Joshi et al, Chen et al, Loane et al),[41-43] failed to observe significant correlations. One reason for the discrepant reports of previous studies could be the variation of "on-medication" or "off-medication" conditions in real-time examination of the PD patients.

There was a significant positive correlation between the mean diffusivity values and duration of the disease (p=0.001) and clinical severity (p=0.001). Kamagata et al, Gattellaro et al, also reported similar findings.[44,45]

The consistent correlation findings from many previous studies imply that nigral FA and MD values could offer an objective assessment of severity of the PD motor dysfunction, when clinical motor measurement is not affected by real-time treatment effects.

## CONCLUSION

The RNFL thickness was significantly lower in PD patients compared to controls. RNFL thickness was negatively correlated with clinical severity.

FA values were significantly lower and MD values were significantly higher in PD patients compared to controls. FA values had a weak negative correlation with clinical severity, and MD values had a statistically significant positive correlation with clinical severity.

OCT and DTI could be used as non-invasive biomarkers for Parkinson's Disease

**Limitations:**

1. The study was a cross-sectional study. A prospective study would have helped to assess the temporal relation between clinical severity and RNFL thickness and the DTI findings.
2. Small study sample.
3. The confounding effect of patients' medication was not considered in the analysis.

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