The evaluation of retinal layers in patients with retinitis pigmentosa with preserved fovea anatomy

Zekeriya Cetinkaya[1], *Osman Ahmet Polat*[2], *Hidayet Sener*[2], *Cagatay Karaca*[2]

ABSTRACT

Purpose: The aim of this study is to detect changes in retinal layers in Retinitis pigmentosa. In this way, it was thought that it could guide researchers in the process of RP treatment development.

Materials and Methods: Twenty-seven RP subjects with preserved foveal anatomy without cystoid macular edema and the same number of healthy people, equal in age and sex, were comprised as the study controls. Macula and retinal layer segmentation was performed by OCT imaging with a Spectralis OCT machine (Heidelberg Engineering, Germany).

Results: In subjects with RP, total retinal thickness was not affected in the 1 mm central area, but it was statistically significantly thinner in all four parafoveal quadrants. In subjects with Retinitis Pigmentosa, ONL, IPL, GCL and RPE were statistically significantly thinner, and OPL and INL were considerably thicker ($p < 0.05$).

Conclusions: In subjects with RP with foveal contour is preserved, there are non-homogeneous changes in the retinal layers and the total retinal thickness decreases in the parafoveal region.

Keywords: Retinitis pigmentosa, OCT segmentation, retinal thickness, foveal anatomy, retinal layers.

INTRODUCTION

Retinitis pigmentosa (RP) is a disease of the retina that is inherited and results in a loss of the retinal photoreceptors and centrally located vision by an advancing deficiency.[1,2] RP can be inherited by mitochondrial, X-linked, autosomal dominant, or autosomal recessive genes.[3] RP is characterized by progressive degeneration and death of rod photoreceptors, so the loss of night vision (nyctalopia) is observed initially. When rod photoreceptors are severely depleted, the death of cone photoreceptors follows, resulting in severe central vision loss.[4]

Histopathological deterioration begins in the peripheral retina, where the rod photoreceptor density is maximum. In the advanced stages of this disorder, a loss of cone photoreceptors also occurs, which causes an extreme loss of vision. The probable cause of the death of the cone photoreceptors is thought to be the result of the loss of rod photoreceptors. Histologically, shortening is observed in the outer segments of the rod photoreceptors first, and shortening in the outer segments of the cones follows in advanced stages.[5-7]

Optical coherence tomography (OCT) is a non-invasive imaging model which allows objective evaluation of the macular structure.[8] Spectral-domain OCT visualizes retinal layers with high-resolution and clearly differentiates layers including choroidal vessels, Bruch's membrane, outer limiting membrane (ELM), elliptical zone (EZ), interdigitation zone (IZ), outer plexiform layer (OPL), inner plexiform layer (IPL), retinal pigment epithelium (RPE) and retinal nerve fiber layer (RNFL).[9,10]

Many studies have reported photoreceptor layer thinning with OCT in RP subjects.[11-14] However, there is no consistency in results for the inner retinal layer in RP subjects.[11,13,15,16] In this study, it was aimed to evaluate retinal

1- MD, Clinic of Ophthalmology, Kayseri State Hospital, Kayseri, Türkiye

2- Asst. Prof., MD, Department of Ophthalmology, Faculty of Medicine, Erciyes University, Kayseri, Türkiye

Received: 23.11.2023
Accepted: 16.02.2024
J Ret-Vit 2024; 33: 106-111
DOI:10.37845/ret.vit.2024.33.16

Correspondence author:
Zekeriya Cetinkaya
Email: zekeriyacetinkaya@hotmail.com.tr

layers with OCT in subjects with retinitis pigmentosa with preserved foveal anatomy, which means all retinal layers are present without displacement in foveal anatomy.

MATERIALS AND METHODS

All performed procedures followed the ethical standards of the performed were following the ethical standards of the Erciyes University Ethics Committee (No: 2020/623 Date: December 16, 2020). Furthermore, this research followed the Helsinki declaration. Informed consent was obtained from each participants in the study.

Twenty-seven RP subjects with preserved foveal anatomy without cystoid macular edema and 27 healthy people, equal in age and sex, were comprised as the study's control group. The study only included one eye of every included subject. The exclusion criteria were as follows: myopia > -6D, hyperopia > +4D, astigmatism > ±3D, intraocular pressure > 22 mm Hg, previous eye trauma, coexisting ocular disease, such as glaucoma, narrow anterior chamber, or senile cataract, which result in inferior views, having had any retinal surgery or having had cataract surgical procedures in the last 12 months, and having used any prescriptions that have been known to have an effect the thickness of the retina, such as steroids and diuretics.

OCT imaging of the macula and retinal layer segmentations were assessed by a Spectralis OCT machine (Heidelberg Engineering, Germany). Image results were considered if they had a signal-to-noise score greater than 25 dB. Analysis did not include decentration of the measurement circle, scans with misalignment, segmentation failure, and those with inadequate illumination or out of focus images.

We determined average thicknesses of layer with ETDRS grid centered at the fovea by the software. The results of retinal layers measurements for each subfield (central 1 mm, superior 1-3 mm, nasal 1-3 mm, inferior 1-3 mm, and temporal 1-3 mm) were automatically segmented (Figure 1). The average thicknesses for ganglion cell layer (GCL), inner nuclear layer (INL), inner plexiform layer (IPL), macular RNFL (mRNFL), outer nuclear layer (ONL, outer plexiform layer (OPL), retinal pigment epithelium (RPE), and inner and outer segments of the photoreceptors (PR) were calculated (Figure 2).

SPSS software (IBM, version 18) was used for the statistical analysis. The Shapiro-Wilk test was used to test the normality of the data. Nominal-ordinal variables were compared with the Pearson chi-square test. Independent variables that were not normally distributed were compared with the Mann-Whitney U test while data that was distributed normally was tested with an independent t-test. Measurements with a p value < 0.05 were taken into account for a statistical significant level in all analyses.

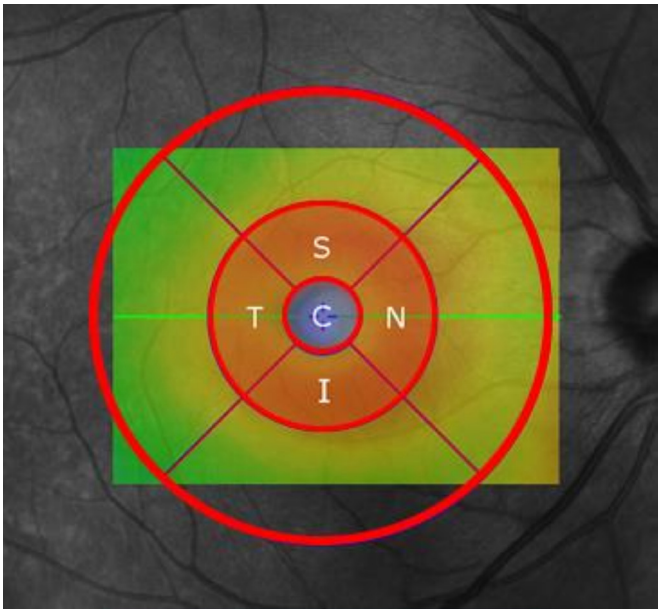

Figure 1: *The subfields of the grid on the macula according to the Early Treatment Diabetic Retinopathy Study (ETDS). Central (C), 1 mm zone, quadrants, 1-3 mm: Inferior (I), Superior (S), Nasal (N), and Temporal (T)*

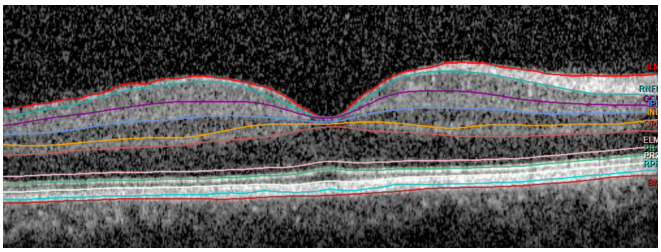

Figure 2: *The layer boundaries, with automatic segmentation are indicated by the abbreviations and lines in color. It was imaged by the spectralis software. ILM: Internal limiting membrane and inner border of the RNFL layer, RNFL: outer border of the retinal nerve fiber layer, GCL: outer border of the ganglion cell layer, IPL: outer border of the inner plexiform layer, INL: outer border of inner nuclear layer, OPL: outer border of outer nuclear layer, ELM: external limiting membrane - outer border of outer nuclear layer, RPE: Retina pigment epithelium, BM: Bruch's membrane.*

RESULTS

The best corrected visual acuity in the retinitis pigmentosa group was 0.4 ± 0.15 on the Snellen chart. In the healthy control group, it was 1.0 ± 0.0. The best corrected visual

acuity was significantly lower in the group with retinitis pigmentosa than in the control group ($p < 0.01$). The control group mean age was 40.3 ± 8.2 (14 males, 13 females), and the mean age of the RP group was 40.7 ± 8.7 (14 males, 13 females), and there were not any statistical variances amongst the RP and control classifications for age or gender ($p = 0.86$), ($p = 0.78$).

Although there was no statistical difference in thickness between the control group and RP patients in terms of centrical region of total retina (1 mm) ($p=0.74$), the total retinal thickness in all subfields of the 1-3 mm was statistically significantly thinner in RP patients compared to the control group ($p\leq0.001$). In the parafoveal areas, the inner retina was observed quite thin in RP patients compared to the control group (Table1).

In subjects with RP, the ONL, IPL, GCL, and RPE were statistically significant thinner, and the OPL significantly thicker. The retinal layers comparison in OCT of the RP subjects and the control group are presented in Table 1.

DISCUSSION

In RP patients with preserved foveal anatomy, decreased thickness was observed in the whole retina and GCL, ONL, and RPE layers in the paracentral regions. In addition, it was observed that the thickness of the ONL and RPE cell layers decreased in the central 1 mm area. However, an increased thickness was observed in the subjects with RP of the INL paracentral and central regions.

Loss of rod photoreceptors causes stress and death in the retinal pigment epithelium in RP.[17-19] Death of rod photoreceptors is followed by degeneration of RPE cells.[20] In the study by Flannery et al., maximum photoreceptor loss was reported in the nasal region in RP patients.[21] In our study, photoreceptor layer thinning was observed in patients with retinitis pigmentosa in the central 1 mm area matched with the group of control subjects. However, there were not any statistical differences in the thickness of the photoreceptor layer in thesuperior, temporal, or nasal parafoveal quadrants. As the disease progresses, the photoreceptor layer gradually thins.[22] This may be because the subjects included in the study were early-stage RP patients with intact foveal morphology.

In a study by Vámos et al., decreased ONL thickness was detected in RP patients.[23] The ONL is a critical biomarker of macular diseases as it is directly related to visual function.[24] Decreased central ONL thickness in RP is associated with a reduction of cone receptors.[25] Different results have been reported on the change in the inner nuclear layer in RP patients. There are reports that the INL thickness, does not change, or decreases slightly.[5,12,26,27] Aleman et al. described a cohort of patients with X-linked RP with a thick inner retina and revealed that this thickening develops secondary to glial cell remodeling due to photoreceptor-induced stress. In addition, they affirmed a highly significant correlation among the thickening of the inner layer of the retina and thinning of the outer retinal layer.[11] In our study, thinning of the ONL layer and thickening of the INL layer were observed in RP patients.

It has been shown in various studies that ganglion cell layers are decreased in RP patients. This decrease was in the parafoveal quadrants.[12,23,26,28] The genes responsible for photoreceptor cell death are thought to exert a direct effect on ganglion cells.[26] In addition, it has been reported that transsynaptic neuronal damage occurs as ganglion cells lose neuronal input due to photoreceptor cell degeneration.[26,28] Since the cone receptor density is high in the central foveal region, we think that photoreceptor loss and transneural degeneration are less than in the parafoveal area. As a result, transneural degeneration is less in the center than in the periphery. In our study, the ganglion cell layer was increased in the central region and decreased in the parafoveal quadrants. However, the clinical course of GCIPL thickening in RP patients is unclear. It is thought that this thickening may result from neural remodeling as retinal degeneration increases.[29,30] Phenomena contributing to GCIPL hypertrophy are thought to be the formation of new synapses and microneuroma, hypertrophy of muller cells, and amacrine and bipolar cell remodeling.[29]

Studies have found that the RNFL layer is thicker in RP patients.[12,23] Unlike the literature, in our study, it was observed that the nerve fiber layer of the retina was thinned in the superior and inferior quadrants, while the temporal and nasal quadrants did not change in RP subjects.

Study Limitations

There are some limitations to our study: RP is a rare disease and our study sample size was small. Therefore, so were the amount of investigated considerations. Furthermore, because the subject and control groups included a low sampling, the power of the data distribution tests might have been low. Early-stage RP patients with preserved retinal anatomy were included in the study to avoid segmentation failure. Therefore, thickness of retinal layers in advanced stage patients were not evaluated. Another limitation is that the genetic mutations of the patients were

Table 1: *Comparison of retinal layers*

Variables	Retinitis Pigmentosa	Control	P	Variables	Retinitis Pigmentosa	Control	P
Total Retina				ONL			
Central	262 (232 - 294)	263 (249 - 281)	0.74	Central	76 (66 - 89)	92 (86 - 99)	**<0.001**
Superior	303 (277 - 314)	347 (334 - 360)	**<0.001**	Superior	58 (50 - 63)	67 (62 - 73)	**<0.001**
Temporal	303 (268 - 315)	332 (319 - 341)	**<0.001**	Temporal	51 (48 - 70)	74 (69 - 78)	**<0.001**
İnferior	304 (278 - 316)	344 (333 - 355)	**<0.001**	İnferior	60 (48 - 65)	65 (62 - 74)	**0.02**
Nasal	306 (277 - 331)	344 (329 - 355)	**<0.001**	Nasal	58 (50 - 63)	72 (65 - 80)	**<0.001**
RNFL				RPE			
Central	13 (11 - 15)	11 (10 - 13)	**0.04**	Central	15 (13 - 17)	17 (16 - 18)	**<0.001**
Superior	18 (14 - 20)	24 (23 - 27)	**<0.001**	Superior	13 (13 - 14)	16 (15 - 18)	**<0.001**
Temporal	18 (14 - 20)	17 (17 - 18)	0.87	Temporal	13 (12 - 15)	15 (14 - 16)	**<0.001**
İnferior	18 (16 - 22)	26 (24 - 27)	**<0.001**	İnferior	14 (12 - 14)	15 (14 - 16)	**0.005**
Nasal	19 (16 - 24)	20 (18 - 22)	0.09	Nasal	13 (12 - 14)	16 (15 - 17)	**<0.001**
GCL				IRL			
Central	17 (13 - 20)	12 (11 - 15)	**0.006**	Central	188 (151 - 212)	174 (160 - 190)	0.26
Superior	21 (15 - 25)	55 (51 - 58)	**<0.001**	Superior	217 (201 - 225)	263 (251 - 280)	**<0.001**
Temporal	25 (17 - 40)	49 (45 - 52)	**<0.001**	Temporal	211 (190 - 232)	250 (237 - 258)	**<0.001**
İnferior	21 (15 - 34)	53 (51 - 58)	**<0.001**	İnferior	231 (201 - 237)	263 (253 - 272)	**<0.001**
Nasal	29 (15 - 32)	52 (49 - 56)	**<0.001**	Nasal	222 (200 - 245)	260 (247 - 273)	**<0.001**
IPL				ORL			
Central	22 (17 - 24)	19 (18 - 21)	0.10	Central	81 (79 - 82)	87 (86 - 91)	**<0.001**
Superior	23 (20 - 30)	43 (42 - 46)	**<0.001**	Superior	82 (78 - 86)	82 (81 - 84)	0.76
Temporal	28 (21 - 38)	43 (41 - 45)	**<0.001**	Temporal	80 (77 - 82)	82 (80 - 85)	**0.048**
İnferior	29 (22 - 39)	43 (41 - 46)	**<0.001**	İnferior	81 (79 - 89)	81 (78 - 83)	0.18
Nasal	33 (21 - 40)	44 (42 - 47)	**<0.001**	Nasal	81 (78 - 87)	82 (81 - 86)	0.19
INL				PRL			
Central	25 (21 - 31)	15 (12 - 18)	**<0.001**	Central	66 (64 - 67)	70 (68 - 74)	**<0.001**
Superior	50 (44 - 60)	41 (39 - 45)	**<0.001**	Superior	69 (64 - 72)	66 (65 - 69)	0.08
Temporal	42 (39 - 50)	37 (34 - 39)	**<0.001**	Temporal	67 (65 - 68)	66 (65 - 69)	0.60
İnferior	51 (49 - 60)	41 (37 - 45)	**<0.001**	İnferior	68 (66 - 72)	66 (64 - 67)	**0.01**
Nasal	50 (47 - 55)	38 (35 - 43)	**<0.001**	Nasal	67 (65 - 72)	67 (65 - 69)	0.29
OPL							
Central	31 (27 - 33)	22 (20 - 27)	**<0.001**				
Superior	41 (38 - 44)	30 (29 - 36)	**<0.001**				
Temporal	37 (34 - 41)	30 (27 - 32)	**<0.001**				
İnferior	38 (35 - 41)	32 (27 - 36)	**0.001**				
Nasal	43 (36 - 48)	31 (29 - 36)	**<0.001**				

RNFL Retinal nerve fiber layer, GCL ganglion cell layer, IPL inner plexiform layer, INL inner nuclear layer, OPL outer plexiform layer, ONL outer nuclear layer, RPE retinal pigment epithelium, IRL inner retinal layer, ORL outer retinal layer, PRL photoreceptor layer, Bold writing indicates that is statistically significant (p value < 0.05).

not evaluated and the relation of the retinal layers with the gene groups was not evaluated.

CONCLUSION

In our study, we showed inhomogeneous changes in retinal layers with OCT in RP patients with preserved foveal contour. The entire retina, ganglion cell layer, ONL, and RPE layer thicknesses decrease in the paracentral area in RP patients, while the INL layer thicknesses increase. There are conflicting results in the literature and therefore, we believe that our findings contribute to the literature.

Ethics

Ethics Committee Approval: Erciyes University Ethics Committee (No: 2020/623 Date: December 16, 2020).

Authorship Contributions

Concept: Z.C., C.K., Design: Z.C., C.K., H.S., O.A.P., Data Collection or Processing: Z.C., C.K., H.S., Analysis or Interpretation: Z.C., H.S., O.A.P., Literature Search: Z.C., O.A.P., Writing: Z.C., H.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

Informed consent: All participating research study subjects gave informed consent.

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