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Human retinal senescence measured histologically: as an aid to clinical diagnosis by ocular coherence tomography

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SUMMARY

Spectral domain optical coherence tomography (SD-OCT) has become an established diagnostic tool for the clinical assessment of retinal pathology and its progression, in OPD setting. The aim of our study was to do in vitro quantification of relevant retinal layers to collect baseline data at different age groups, against which OCT findings can be interpreted. Thirty eyeballs (20-99 years) were used to study the retinal nerve fibre layer (RNFL), ganglion cells and inner plexiform layer (GC+IPL) and outer nuclear layer (ONL) thickness using V-Test software, 198035 on the H&E stained histological specimens. Mean thickness of these retinal layers was studied. To estimate the decrease from the optimal stage, absolute percentage decline (APD) was calculated for each decade. The mean thickness of RNFL at was 77.8 µm, 77.1 µm, 73.6 µm, 70.6 µm, 69.2µm, 54.1µm, 36.5µm 26.8µm from 3rd to 10th decade respectively. Significant APD of 30% was evident between 7th and 8th decades. APD graphs for RNFL and GC+IPL were almost parallel to each other. The absolute percentage decline (APD) for the thickness of ONL was 0.2%, 4.6%, 13%, 35%, 60%, 62% and 64% for 4th, 5th, 6th, 7th, 8th, 9th and 10th decade respectively. This study has provided normative base line histological data for ready reference. Decade wise changes in thickness of different layers can be used by ophthalmologist to differentiate senescent from pathological changes and to monitor progression of disease.

Key words: Retina – Nerve fibre layer – Ganglion cell and inner plexiform layer complex – Outer nuclear layer – Optical coherence tomography

INTRODUCTION

Various common retinal disorders like Glaucoma, Retinitis pigmentosa are clinically characterised for diagnosis as well as for disease progression on the basis of thickness of different retinal layers. A progressive change in retinal nerve fibre layer (RNFL) thickness is used for the evaluation of glaucoma. Recent studies have concluded that thickness of ganglion cell layer and inner plexiform layer combined (GC+IPL) can also be used for diagnosis of glaucoma (Shaw and Weber, 1983; Celebi and Mirza, 2013; Kim et al., 2015a, b). Seol et al. (2015) have found GC+IPL thickness to be more accurate than the RNFL thickness for glaucoma detection in myopic eyes. Thickness of the outer nuclear layer (ONL) has been correlated with cone density values in patients with retinitis pigmentosa (Mwanza et al., 2011). But most of these pathognomonic changes, in lesser degree, are also seen in normal population with aging.

With the advent of optical coherence tomography (OCT) especially spectral domain OCT (SD-

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OCT) these morphometric criterions are being routinely employed in ophthalmic practice. The SD-OCT gives high definition images of retinal cross section, comparable to retinal excisional biopsy (Chui et al., 2012). There is a key gap in this process as morphometric data pertaining to normal aging which is required for reference to improve the diagnostic specificity is not readily available in the literature. Most of the studies are radiology based and focus on retinal nerve fibre layer thickness (Lai et al., 1978; Shaw and Weber, 1983; Mwanza et al., 2011; Celebi and Mirza, 2013; Chen et al., 2013; Danesh-Meyer et al., 2015; Francoz et al., 2014; Kim et al., 2015a, b; Lujan et al., 2015; Menghini et al., 2014; Park et al., 2015; Seol et al., 2015). There are a few histology based studies; mostly on animals & with small sample size (Sung et al., 2009; Chui et al., 2012; Lee et al., 2012; Begum et al., 2014), but to best of our knowledge there is no study available which deals with detailed morphometric data from different layers of human retina and covers the whole adult age spectrum. Knowledge of age related changes in thickness of various retinal layers in normal population is essential, as this age related normative values can be used as reference point for diagnosis of pathological conditions, based on measurement of retinal thickness by OCT. Therefore the present study was planned to measure various layers of human retina at different age groups from 3rd to 10th decade of life.

MATERIALS AND METHODS

Material

This is a cross sectional study on 30 fresh adult eyes balls obtained from unembalmed cadavers (received through body donation programme running in the Department of Anatomy and from eye donation programme in the Institute), with age ranging from 20 years to 99 years (Table 1). The average time from death to the fixation process was 2 hours with maximum of 4 hours in three cases. Specimens with history of ocular disease, ocular trauma, hypertension or diabetes were excluded from the study. Ethical clearance was taken from the institutional ethical committee.

Method

Eye balls from the cadavers were enucleated with the help of an enucleation spoon. Each eye ball was sectioned parallel to the equator nearer to the posterior pole and fixed in 10% buffered formalin. This was followed by paraffin embedding, block making and sectioning. The sections for haematoxylin and eosin staining (H&E) were 5 micrometers thick. The H&E staining were done by regular protocol.

Morphometry (Fig 1): Each specimen was studied and micro photograph was taken with the help of Ci-L Pentahead Nikon microscope (700857) with camera (MC 30). The photographs were transferred to the computer. The thickness of various retinal layers were taken on photographs taken at 100x magnification with the help of V-Test software, 198035 (Russia, St.-Petersburg, 2009). The measurements were taken perpendicular to the surface of inner limiting membrane of the retina. For statistical purpose the mean value with standard deviations and range, for every parameter was calculated for each age group in 30 specimens. For this purpose we have also calculated the absolute percentage decline (APD) from the optimum stage of 3rd decade (as explained later). The layer thickness at 3rd decade was taken as the reference point or optimal stage. To estimate the decrease from the optimal stage, absolute percentage decline (APD) was calculated for each decade by subtracting the layer thickness of each decade from the thickness at 3rd decade.

RESULTS (FIG 2, FIG. 3)

1. Retinal nerve fibre layer (RNFL)

The mean thickness of the retina nerve fibre layer thickness was found to be 60.6 µm. The mean thickness of RNFL at 3rd decade was 77.8 µm,

Table 1. Decade and age wise distribution of the studied specimens

Decade (n)	Third (2)	Fourth (2)	Fifth (2)	Sixth (3)	Seventh (5)	Eighth (5)	Ninth (6)	Tenth (5)
Exact age	21 F	34 M	43 M	58 M	62 F	71 M	81 F	90 F
	22 M	38 M	49 M	60 M	67 M	74 M	84 F	91 F
				60 F	67 F	76 M	85 M	92 F
					68 F	77 F	85 F	95 M
					70 M	80 M	87 F	95M
							90 M	

M = Male; F = Female.

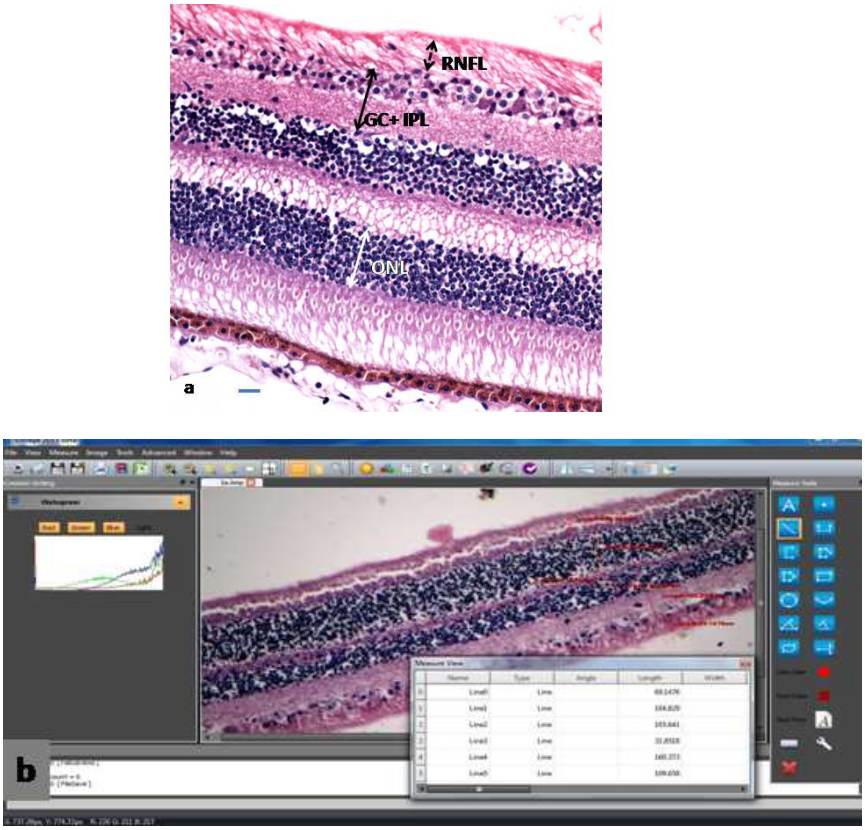

Fig. 1. (a) H & E stained section of retina seen at 40x magnification. Scale bar = 5 µm. **(b)** Photograph depicting the method used for the morphometry of various retinal layers with the help of V-Test software, 198035.

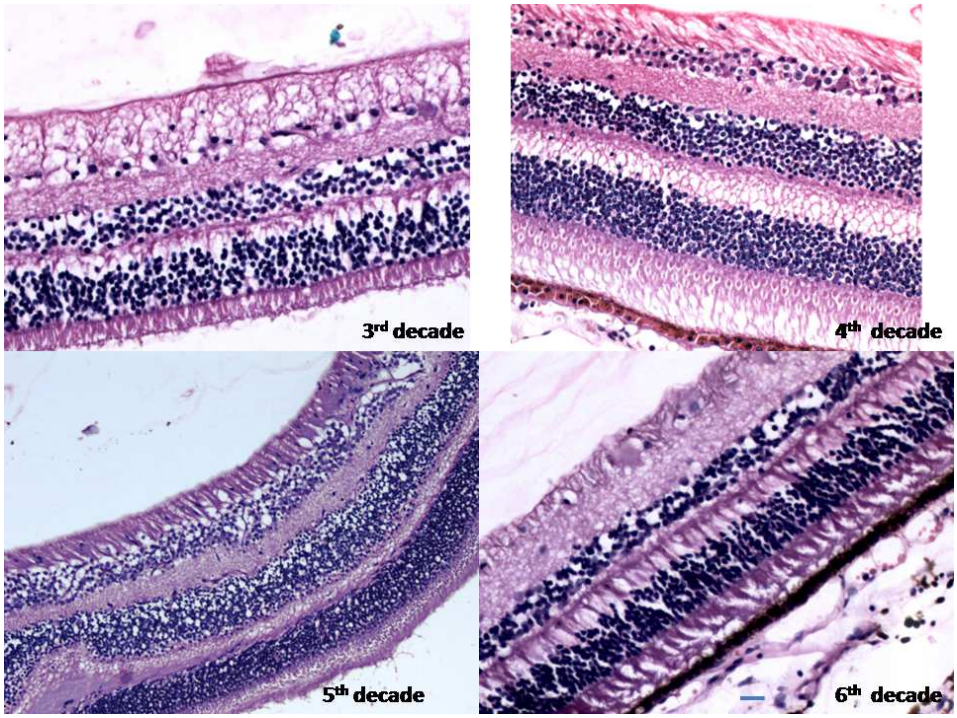

Fig. 2. Different layers of retina at 3rd, 4th, 5th and 6th decades. All sections are H & E stained and seen at 20x magnification. Scale bar = 10 µm.

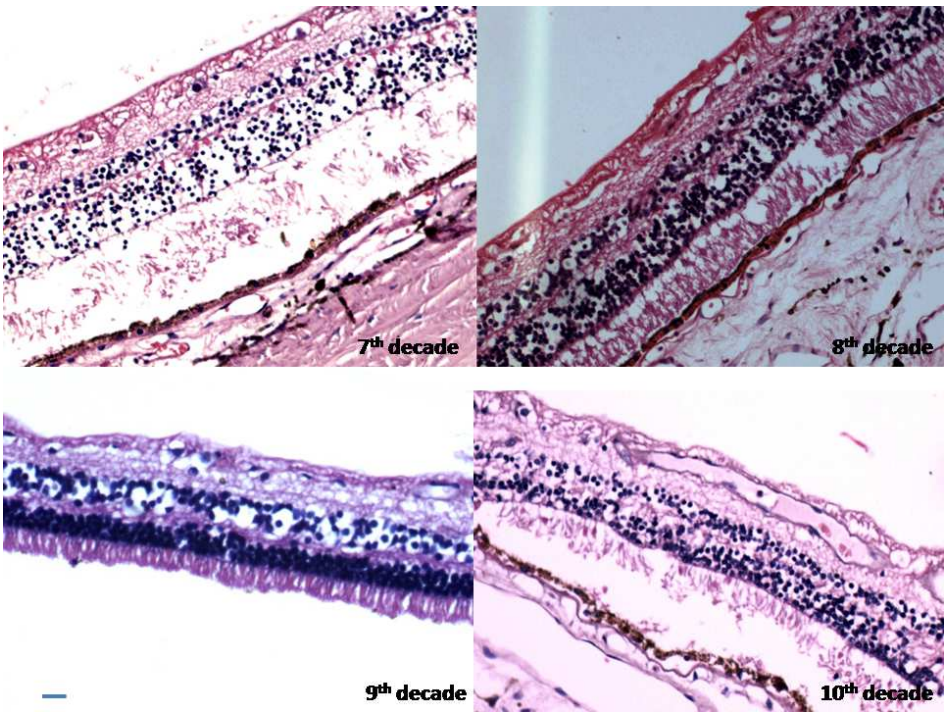

Fig. 3. Different layers of retina at 7th, 8th, 9th and 10th decades. All sections are H & E stained and seen at 20x magnification. Scale bar = 10 µm.

77.1 µm at 4th decade, 73.6 µm at the 5th decade, 70.6 µm at 6th decade, 69.2µm at 7th decade, 54.1µm at 8th decade, 36.5µm at 9th decade and 26.8µm at 10th decade. Percentage decline in each decade was calculated on the basis of the difference in the thickness from the preceding decade. It was found to be 0.77%, 4.54%, 4.08%, 1.98%, 21.82%, 32.53% and 29.32% at 4th, 5th, 6th, 7th, 8th, 9th and 10th decade respectively. The RNFL thickness at 3rd decade was taken as the reference point or optimal stage. To estimate the decrease from the optimal stage, absolute percentage decline (APD) was calculated for each decade by subtracting the RNFL thickness of each decade from RNFL thickness at 3rd decade. The absolute percentage decline (APD) was 0.77%, 5.4%, 9.26%, 11.05%, 30.46%, 53.09% and 65.55% for 4th, 5th, 6th, 7th , 8th, 9th and 10th decade respectively.

2. Ganglion cell layer and Inner plexiform layer (GCL+ IPL)

Thickness of these two layers was taken together. The mean thickness of GC+IPL was 94.05µm. Age wise thickness of these two layers was found to be 119.6 µm, 120.1 µm, 109.1 µm, 104.7 µm, 99.7 µm, 72.3 µm, 64.9 µm and 62 µm at 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th decade respectively. Similar to RNFL, absolute percentage decline (APD) for each decade was also calculated for GCL+IPL. In the 4th decade the thickness was found to be 120 µm, same as in 3rd decade. From this value the APD was 9.2%, 12.75%, 16.9%, 39.75%, 45.9% and 48.3% in 5th, 6th, 7th, 8th, 9th and 10th decade respectively.

3. Outer nuclear layer (ONL)

The mean thickness of the outer nuclear layer thickness was found to be 112.5 µm. The mean thickness of ONL at 3rd decade was 160.4 µm, 160.1 µm at 4th decade, 153 µm at the 5th decade, 138.9 µm at 6th decade, 103.8µm at 7th decade, 64.4µm at 8th decade, 61.1µm at 9th decade and 58.4 µm at 10th decade. The absolute percentage decline (APD) for the thickness of ONL was 0.2%, 4.6%, 13%, 35%, 60%, 62% and 64% for 4th, 5th, 6th, 7th, 8th, 9th and 10th decade respectively.

DISCUSSION

In recent years, OCT has emerged an important tool which relies on direct measurements of retinal layer thickness for diagnosis of diseases such as glaucoma and retinitis pigmentosa. Population and age based data related to changes in the thickness of different retinal layers in therefore necessary.

Death of ganglion cells is the common final pathway event in glaucoma pathophysiology leading to progressive RNFL thinning, as RNFL is formed by the efferent axons of the ganglion cells. RNFL thickness variation over time is used for the quantitative evaluation of the glaucoma. On the

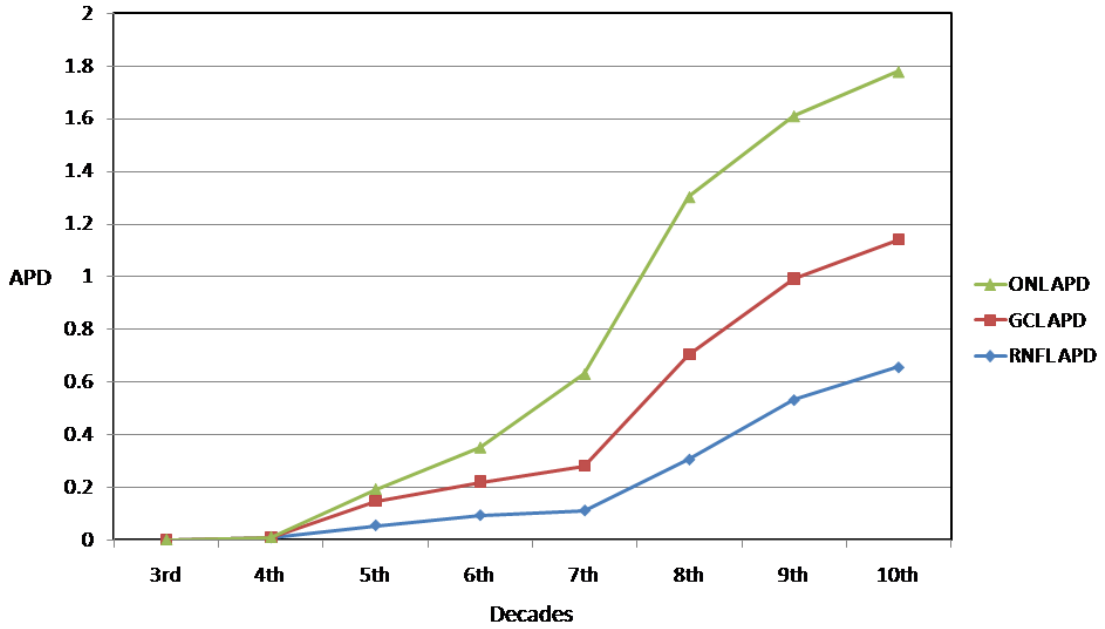

Fig. 4. Graphical representation of decade wise absolute percentage decline (APD) in the values of Outer nuclear layer (ONL), Ganglion cell and Inner plexiform layer (GCIPL) and Retinal nerve fibre layer (RNFL). X axis denotes decades from 3rd to 10th while Y axis denotes the APD; the shown values are in %.

other hand, progressive retinal nerve fibre layer (RNFL) thinning is also associated with normal aging. As progressive RNFL thinning is caused by both physiologically in aging and pathologically in the glaucoma, it is important to assess the rate of RNFL thinning associated with age for the accurate assessment of the damage inflicted by the disease process. Recent OCT based studies have reported that RNFL thickness decrease from 99.04 ± 4.20 µm in 20 to 29 year age group to 89.60 ± 4.73 µm in 60 to 79 year age group with 0.365 µm decrease in overall mean RNFL thickness per year increase in age (Chen et al., 2013; Lujan et al., 2015). In the present study we found marked reduction in mean RNFL thickness with age, from 77.8 µm at second decade to 26.8 µm at 10th decade. In the present study the RNFL thickness was found to be less than those reported by the OCT based study. The reason could be the shrinkage because of formalin fixation and processing for histological preparation. To overcome this handicap we have calculated the percentage decline per decade and absolute percentage decline. Till 7th decade annual decline in the RNFL thickness was nominal, followed by significant decline of 22% between 7th and 8th decade; 33% decline between 8th and 9th decade and 30% decline between 9th and 10th decade (Table 2). These values, in conjunction with other factors, can help in prognostication of visual acuity with age. First significant absolute percentage decline (APD) of 30% was evident between 7th and 8th decades, while the thickness was reduced to half in the eighties and less than 40% in nineties (Fig 3, Fig 4). Given the importance of RNFL thickness as a means for quantifying glaucomatous damage with the help of SD-OCT, the paucity of high- quality data on retinal nerve fibre thickness is a drawback (Lujan et al., 2015). This data will be helpful in diagnosis and prognosis of Glaucoma. Moreover there are recent reports in literature where RNFL thickness as measured by OCT has been used to predict the visual outcome in pituitary tumours and parachiasmal meningioma. Patients with normal RNFL thickness before surgery were found to be more likely to have visual improvement after surgery than patients with a thin RNFL (Francoz et al., 2014; Seol et al., 2015). The normative data for RNFL thickness for each decade generated in the present study can be used for reference in monitoring such patients.

With technological advance in SD-OCT it is now possible to measure ganglion cell layer and inner plexiform layer (GCL+ IPL) thickness at macula with the help of macular ganglion cell analysis algorithm (Lai et al., 1978; Kim et al., 2015). This has led to increased interest in GCL+ IPL thickness to diagnose and monitor glaucoma. Kim et al. (2015b) in retrospective, longitudinal study of 109 clinically stable open-angle glaucoma patients studied both RNFL and GCIPL and concluded that GCIPL can be considered as an effective means of monitoring glaucomatous progression in macula. Similarly Begum VU et al. (2014) in their cross- sectional study of 53 eyes of normal subjects and 83 eyes of glaucoma patients concluded that the diagnostic ability of GCIPL parameters as seen by HD-OCT, was similar to that of ONH (optic nerve head) and peripapillary RNFL

parameters in perimetric glaucoma. Seol BR et al. (2015) suggest that macular GCIPL thickness can be used as an early indicator of glaucomatous structural damage in preperimetric glaucoma (PPG). Moreover they conclude that the macular GCIPL thickness was the best PPG-detection parameter for myopic eyes as both ONH and RNFL parameters are not dependable in myopics, as ONH thickness varies greatly in myopics and RNFL thickness is less than the non-myopic eye. Many studies have reported high reproducibility of GCIPL data in both short-term and long term (Park et al., 2014; Kim et al., 2015a, b). All these studies emphasising the clinical relevance of GCIPL, underlines the importance of the normative GCIPL data collected in the present study. A significant decline (APD) of 40% in GCIPL thickness was noted in 8th decade with 2% per decade absolute decline (APD) in 9th and 10th decades (Table 2). On comparing the APD graphs for the RNFL and GC+IPL (Fig 4), both were seen to be running almost parallel to each other. This fact further corroborates existing evidence that GC+IPL thickness can also be used to diagnose and monitor glaucoma.

Outer nuclear layer thickness (ONL) has been widely used as a marker for photoreceptor numbers. Menghini et al. (2014) have reported that ONL thickness as measured using OCT is directly correlated with cone density measures in normal as well as in retinitis pigmentosa patients. Retinal degeneration characterized by atrophy of photoreceptors and partial degeneration of neurons in the outer nuclear layer has been reported in the rat retina. These changes are attributed to low-intensity light damage related to physiological aging. Lai et al. (1978) ONL thickness is an easily obtainable indirect parameter to represent cone density values both in diseased and healthy individuals. Chui et al. (2012) evaluated the relationship between cone photoreceptor packing density and outer nuclear layer (ONL) thickness. They concluded that the individual differences in cone packing density and ONL + Henle fibre layer thickness are consistent with aging changes, indicating that normative aging data are necessary for fine comparisons in the early stages of disease or response to treatment. ONL thickness measurements are obscured in standard optical coherence tomography (OCT) images because of Henle fibre layer (HFL). The ONL and HFL can be better differentiated with optical contrast of the directional OCT. Distinguishing these individual layers can improve assessment of disease progression (Menghini et al., 2014). In the present study, on calculating the APD in the thickness of the ONL, first significant decline of 13% was seen at 6th decade. At the 7th decade 35% reduction from the optimal stage occurred. This decline stabilised at 60-64% from 8th to 10th decades (Fig 4). The age wise morphometric data from cadaveric eyeballs will fill the key gap in the current knowledge as almost all the studies available in literature are OCT based.

The absolute percentage decline for RNFL, GC+IPL and ONL is given for each decade (Table 2). The plotted graphs indicate the decade wise decline trend (Fig 4). We propose that any decline value falling to the left of the graph for the given decade, should be treated as pathological and investigated further, in the course of monitoring for glaucoma (RNFL, GC+IPL) or retinitis pigmentosa (ONL).

The aim of our study was to do in vitro quantification of those retinal layers which are significant in diagnosing and prognosis of common retinal diseases. Spectral domain optical coherence tomography (SD-OCT) has become an established diagnostic tool for the clinical assessment of retinal pathology and its progression in adults in OPD setting. OCT provides opportunity to see the retinal microanatomy in vivo. This has renewed the interest to have the base line histological data on non-pathological age related changes for ready reference. Decade wise changes in morphometric data can be used by ophthalmologist to differentiate senescent from pathological changes and to monitor progression of disease.

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