# New Trends for Early Diabetic Retinopathy Diagnosis

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Abstract: Diabetes Mellitus is one of the most common chronic diseases in the world and is a critical public health problem that could even be considered a pandemic. The diabetic retinopathy is the leading cause of blindness in adults. Diabetic retinopathy is now considered to be a new neurodegenerative disease. In fact, retinal neurodegeneration is present before any microcirculatory abnormalities can be detected in ophthalmoscopy. Functional studies documenting electroretinogram abnormalities, loss of dark adaptation, contrast sensitivity and colour vision and abnormal microperimetry that occur before any vascular abnormality. Novel imaging optical devices have allowed that this pre-vascular damage to be quantified in a non-invasive and reproducible way with retinal layer and choroidal thickness measurement.

## 1 INTRODUCTION

Diabetic retinopathy (DR) is the leading cause of legal blindness among working-aged adults in the United States (Klein, 2007). Of the 415 million diabetic patients worldwide in 2015, over one-third will develop DR in their lifetime (International Diabetes Federation (IDF), 2015).

The RETINODIAB study, an epidemiologic study that investigated the prevalence and progression rates of DR based on a national screening community program in Portugal, identified a 16.3% prevalence rate of DR and a 4.6% incidence rate of any DR within the first year in diabetic patients without retinopathy at baseline (M. Dutra Medeiros *et al.*, 2015; Marco Dutra Medeiros *et al.*, 2015).

The International Clinical Classification of DR is based on the observation of microvascular retinal changes (‘Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group.’, 1991). However, diabetic neuroretinal degeneration has been demonstrated in histological studies and through the measurement of functional loss with a number of functional tests, including contrast vision, color vision, visual field, dark adaptation and electroretinogram. These retinal neurodegenerative changes include apoptosis of several populations of retinal cells (e.g., photoreceptors, bipolar cells, ganglion cells and astrocytes) with consequent effects on the thickness of different retinal layers in the earliest stages of DR or when DR cannot be detected by ophthalmologic examination (Barber *et al.*, 1998; Carrasco *et al.*, 2007, 2008; Garcia-Ramírez *et al.*, 2009). Furthermore, it has been hypothesized that changes in the choriocapillaris may precede the development of DR (Nagaoka *et al.*, 2004). However, the relationship between DR and diabetic choroidopathy remains unclear.

Recently, optical coherence tomography (OCT) has been introduced into clinical practice as the most non-invasive and objective method to visualize the retina, showing an amount of detail that resembles histological specimens (Fischer *et al.*, 2009; van Dijk *et al.*, 2011). Initially, OCT was applied to detect complications of DR (edema macular or epiretinal membrane) (Ceklic, Maár and Neubauer, 2008). Later on, it allowed to perform quantitative and qualitative measurements of retinal thickness and segmentation of all intraretinal layers (van Dijk *et al.*, 2009, 2010, 2012; Vujosevic and Midena, 2013; Tavares Ferreira J *et al.*, 2016), Figure 1. The new Spectralis Spectral

Tavares Ferreira J. and AbegÃ¢o Pinto L.
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Domain-OCT automatic segmentation software demonstrated excellent repeatability and reproducibility of each of the eight individual retinal layer thickness measurements (Ctori and Huntjens, 2015). Potentially, OCT might detect early retinal changes, and thus help define which diabetic patients may be at-risk to develop DR. Ultimately, it could be used to plan preventive therapy before the development of vascular lesions detectable by ophthalmoscopy (Simó and Hernández, 2014). However, up until now, the smaller scale, mostly pilot studies or only focusing on specific retinal layers on this topic in OCT image analysis did not show a temporal relationship between DM duration or arising DR and the changes observed in retinal layers.

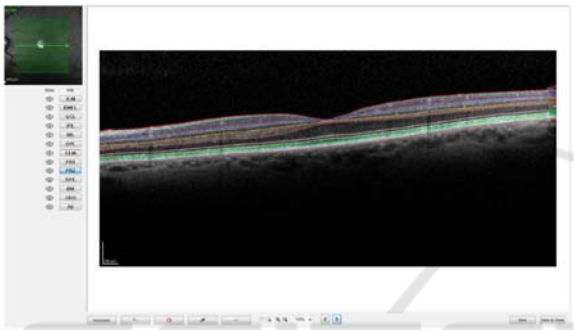

Figure 1: Retinal layer segmentation. Figure adapted to (Tavares Ferreira J, Alves M, Dias-Santos A, Costa L, Santos BO, Cunha JP, Papoila AL, 2016).

This review aims to summarize recent literature concerning retinal structure changes in diabetic patients and its relationship with diabetic retinal disease progression.

## 2 OPTICAL COHERENCE TOMOGRAPHY IN EARLY DIAGNOSIS OF DIABETIC RETINOPATHY

Several studies using Spectral Domain (SD)-OCT, showed a decreased retinal nerve fiber layer (RNFL) or (ganglion cell layer) GCL thickness in diabetic patients without DR (Vujosevic and Midena, 2013; Chhablani *et al.*, 2015; Carpineto *et al.*, 2016). However, Hille van Dijk et al. did not find differences in any inner layer thickness between non-diabetic and type 1 or type 2 diabetic patients even without DR (van Dijk *et al.*, 2010, 2012). Nevertheless, different SD-OCT devices were used (Cirrus, Topcon or Nidek) and the diabetic patient samples were very small (30 (Vujosevic and Midena, 2013), 20 (Chhablani *et al.*, 2015), 19 (van Dijk *et al.*, 2010) or 39 (van Dijk *et al.*, 2012) patients to perform a reliable multivariable analysis. Only Carpineto *et al.* studied 131 type 2 diabetic patients without DR using Cirrus SD-OCT, and identified a reduced ganglion cell-inner plexiforme layer (GC-IPL) and RNFL thickness compared with healthy controls (Carpineto *et al.*, 2016). Vujosevic *et al.* studied both inner and outer layers but in opposition to this work they did not find any differences in the retinal pigment epithelium (RPE) and photoreceptor (PR) layers thickness. However, these authors have studied the RPE and PR layers together not individualizing them in two different layers.

Tavares Ferreira *et al*, used SD-OCT to compare the retinal layers thickness between non-diabetic subjects and type 2 diabetic patients without DR and with different Diabetes Mellitus (DM) duration (Tavares Ferreira J *et al.*, 2016). In their multivariable regression models, after adjusting for age, gender, intraocular pressure (IOP) and axial length, and correcting for multiple testing, no difference in the overall retinal total (RT) thickness throughout the Early Treatment Diabetic Retinopathy Study (ETDRS) areas was found. Interestingly, the patterns of layer distribution were not the same in the two samples. The PR layer was the most consistent finding, with a smaller thickness in diabetic patients when compared to their non-diabetic controls, Figure 2.

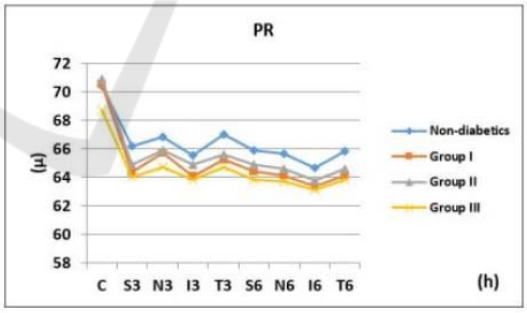


Figure 2: Photoreceptor layer thickness in all groups, determined automatically by SD-OCT in nine ETDRS areas in the macula. Figure adapted to (Tavares Ferreira J, Alves M, Dias-Santos A, Costa L, Santos BO, Cunha JP, Papoila AL, 2016).

Nevertheless, the pattern of thickness in this layer differs with disease duration. Once stratified diabetic patients according to this parameter, the thinner layers could be found in patients with both an early (group I) and longer known diabetes diagnosis (group III) ($p<0.001$). On the other hand, the thinning in PR in diabetic patients with moderate duration (group II)

did not reach statistical significance when compared to the healthy controls. The remaining layers (outer nuclear layer - ONL, outer plexiform layer - OPL, inner nuclear layer - INL and GCL) showed an overall tendency towards a thicker layer in diabetic retinas when compared to non-diabetic patients, but did not reached statistical significance (Tavares Ferreira J *et al.*, 2016).

PR layer was not uniform throughout disease duration. This could be interpreted as a temporary cellular swelling due to a number of reasons, ranging from the diabetic induced hypoxia (Kern and Berkowitz, 2015), oxidative stress with increased generation of superoxide and other reactive oxygen species in the retina (Du *et al.*, 2013) which induces the release of pro-inflammatory molecules and changes in retinal vasculature. Ultimately, the continuous cellular swelling is known to lead to a cellular atrophy (Kern and Berkowitz, 2015), potentially explaining the thinnest PR layer in the patients with longer disease duration. This non-linear behaviour is important as it can explain the contradictory results in this field, as each study may be recruiting patients with a different disease duration. Additionally, it could be clinically relevant as studies have suggested the importance of the PR layer in the development of DR, loss of PR reduced the severity of vascular degeneration in DR (Arden, 2001; De Gooyer *et al.*, 2006). Further studies would be needed to interpret such findings.

These same authors did a longitudinal study, based on the baseline study referred in which the aim was to evaluate which diabetic patients without DR would develop DR after one year and to use SD-OCT to detect changes in retinal and choroidal layers over a period of one year (Tavares Ferreira J *et al.*, 2016). These 125 type 2 diabetic patients without DR showed that after one year, independent of the development of DR, the choroidal thickness (CT) increased between 10 and 17 µm ($p<0001$ to 0003), and there was a decrease in the GCL (I3 and N6 sectors), IPL (S6 and N6 sectors), INL (T6 and N6 sectors), OPL (S6 sector) and overall RT (S3, N3, I3, S6 and T6 sectors) ($p<0.001$). Interestingly, in this study, the variable retinopathy was negatively associated with the overall RT (central, S3, T3 I3 and N3 sectors), ONL (T6 and I6 sectors) and PR layer (N6 sector). In the span of just one year, the presence of DR decreased the overall RT in the studied locations between 13.04 and 16.63 µm (Tavares Ferreira J *et al.*, 2016).

Overall these results may be explained by a process of inflammation which accompanies or precedes the early cell apoptosis of the DR. Thus, before there is a thinning of the different inner retinal layers, there is a significant thickening compared to non-diabetic patients. When some cells are in apoptotic stage and others in inflammatory phase probably we will not find any significant differences in the thickness of the retinal layers. Further, when analysing the RPE layer, a significant association between the RPE thickness and the CT was noticed, so it is important to include the variable CT in the regression models. Ferreira *et al.* found that the CT in diabetic patients without DR was increased in comparison with non-diabetic subjects, (Ferreira *et al.*, 2015) and since the choroid supplies the RPE the referred association between CT and RPE would be expected.

This last study had some limitations (Tavares Ferreira J *et al.*, 2016). Firstly, despite including 125 diabetic patients without DR, when divided into groups according to diabetes duration, their sample sizes became small. However this is the first study with a considerable sample that splits the diabetic patients according to disease duration, and yet finding differences in these subgroups compared to non-diabetic subjects. Secondly, retinal measurements were done with automatic software. The ideal retinal layers segmentation is one that involves automatic segmentation with supervision and manual correction when necessary. In this way, a manual correction was performed when the segmentation was inaccurate by an ophthalmologist masked to the patients' diagnosis. Thirdly, all diabetic patients had type 2 DM meaning that the onset of diabetes was self-reported and could thus be underestimated.

# 3 CONCLUSIONS

In conclusion, diabetic patients without DR have a thinning of some retinal layers (inner retinal and PR layers), when compared to a non-diabetic group. There are early changes in retinal layers of diabetic patients even without clinical signs of DR that probably correspond to an inflammatory and apoptotic process of the retina as neurovascular unit.

In diabetic patients without DR at the one-year follow-up point, was observed overall thickening of the choroid and decreases in the thickness of the inner retinal layers (GCL, IPL and INL) and overall retinal thickness (RT). Thus, when patients develop DR, the choroid begins to decrease along with the overall RT and PR layer thickness.

The OCT is a new technology that allows the diagnosis of early retinal and choroid structural

changes of diabetic patients before the onset of diabetic retinopathy.

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