Alterations in Retinal Layer Metrics and Their Association with Visual Acuity in Diabetic Retinopathy

BY

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THESIS

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SUMMARY

Diabetic Retinopathy (DR) is an eye disease in people with diabetes and the leading cause of vision loss in working-age adults. DR causes alterations in retinal anatomy, both in the neural retina and in the retinal vasculature, resulting in visual acuity (VA) loss. Regular assessment and monitoring disease progression along with timely therapeutic intervention are necessary to prevent vision loss due to DR.

Distinct previous studies have reported changes in retinal layer thickness due to DR and their association with VA, as well as changes in retinal layer reflectance and organization. However, concurrent assessment of these metrics and their relationship with VA has not been reported. In the current study, our primary aim was to determine the associations of retinal layer thickness, retinal layer reflectance, and retinal layer disruption with VA. Secondly, to elucidate alterations in these metrics over time, in subjects stratified by treatment.

Quantitative retinal metrics were obtained from optical coherence tomography (OCT) images in 149 diabetic patients, categorized into three groups: no DR (N=50) non-proliferative DR (NPDR; N=59) proliferative DR (PDR; N=40). In a follow-up study, 23 of these patients were imaged at a second visit, who were grouped based on treatment and changes in retinal layer metrics over time were evaluated. The OCT images were analyzed using a semi-automated

image segmentation software to identify 7 different retinal layers and generate en face thickness maps and reflectance images.

ANOVA identified significant differences in thickness of the central subfield (CST) among the three subgroups of patients (P=0.01). VA and thickness of three retinal structures: CST, INL and OPL were correlated significantly ($\rho>0.24$; $P<0.001$), such that increased thickness was associated with reduced acuity. VA and NFL reflectance were correlated significantly ($\rho=-0.24$; $P=0.003$), such that subjects with higher reflectance had better VA. Lower OSL reflectance was correlated with lower VA ($\rho=-0.29$; $P<0.001$). Subjects with disruptions in the INL and ONL had lower VA compared to subjects with no disruptions in these interfaces ($P<0.001$). In the follow-up study, RPE and OSL thickness were significantly different between two visits.

These results indicate that assessment of specific retinal structures may be helpful for monitoring visual outcome due to DR. Similarly, reflectance alterations, which are not commonly evaluated, may provide additional important information. Finally, changes in retinal thickness of select retinal layers can be observed over a relatively short time in DR, with significant thickening observed in the RPE and OSL

CHAPTER 1

INTRODUCTION

1.1 MOTIVATION

Diabetic Retinopathy (DR) is an eye disease in people with diabetes and the leading cause of vision loss in working-age adults. It is estimated that the global prevalence of diabetes is expected to be 4.4% by 2030 for all age groups across the world, resulting in an increase in the incidence of DR and the associated vision impairment (1; 2). This complication of diabetes affects the retina, the light-sensitive tissue in the back of the eye, and its vasculature, resulting in loss of visual acuity (VA) (3). Early screening, regularly monitoring the progression of the disease and timely therapeutic intervention can prevent vision loss due to DR. Optical coherence tomography (OCT) is an imaging technique that enables visualization of retinal tissue with high resolution. Several studies have reported that OCT enables retinal thickness measurement with high accuracy (3; 4). In addition to retinal thickness measurements, a number of other structural metrics can be derived by OCT including retinal reflectance and retinal layer interface disruption/disorganizarion. Although retinal anatomical changes and associations with visual acuity (VA) have been reported in patients who have DR, the metrics were assessed independently in separate groups of patients (5; 6; 7). This thesis presents a

concurrent assessment of alterations in retinal layer metrics of thickness, reflectance, and interface disruption and their relationship with VA, which can provide a consolidated means for better assessment of DR.

1.2 DIABETIC RETINOPATHY (DR)

Diabetic Retinopathy is associated with damage to the blood vessels in the retina. It can cause blood vessels in the retina to leak fluid or hemorrhage, distorting vision. Accumulation of fluid in the retinal tissue results in retinal thickening and vision loss. In its most advanced stage, new abnormal blood vessels proliferate on the surface of the retina, this can lead to scarring and cell loss in the retina. DR is classified into non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR), which is marked by the growth of new blood vessels. NPDR is sub-divided into mild, moderate and severe based on presence of aneurysms, intra-retinal hemorrhages, and venous beading (8; 9). A consequence of DR, diabetic macular edema (DME), is the build-up of fluid in the macular region of the retina. Although it is more likely to occur as DR worsens, DME can happen at any stage of the disease. As DR progresses, many changes occur in the retina without visual symptoms. Hence, early diagnosis and adequate treatment is necessary.

Anti-vascular endothelial growth factor (anti-VEGF) treatment is one of the therapeutic methods to prevent vision loss due to DR. Anti-VEGF treatments are given by injections into the eye, which restrict the growth of new blood vessels and reduce edema. This reduces

scarring and damage to the retina, which in turn can help preventing further vision loss due to PDR or DME and may also improve vision in some people (10).

1.3 BACKGROUND

1.3.1 Retinal Structure:

The retina is a highly organized structure that consists of three layers of nerve cell bodies (outer nuclear layer, inner nuclear layer, and ganglion cell layer) and two layers of synaptic connections (inner and outer plexiform layers). These layers can be visualized using histological sections as well as non-invasive OCT imaging. Several additional structures can be visualized using modern high-resolution OCT imaging. The inner-most structure of the retina visible by OCT is the inner limiting membrane, which is a basement membrane enriched with muller glia cells. Below that lies the nerve fiber layer (NFL), which includes ganglion cell axons and optic nerve rises from here. Next is the ganglion cell layer (GCL) that consists of ganglion nuclei and dislocated amacrine cells, followed by the inner plexiform layer (IPL), the synaptic site between axons of the bipolar cell and the dendrites of the amacrine and ganglion cells. The inner nuclear layer (INL), is composed of the cell bodies and nuclei of horizontal, bipolar, amacrine cells, and muller glia cells. The outer plexiform layer (OPL) is made of projections of cones and rods which have synaptic connections with horizontal and bipolar cell dendrites. The outer nuclear layer (ONL), contains cell bodies of cones and rods. The photoreceptor layer

(OSL) is composed of the inner and outer portions of the cones and rods. The outer layer visible by OCT is the retinal pigment epithelium (RPE).

1.3.2 Role of OCT in measuring structural alterations of the retina:

OCT is a standard clinical technique that plays a key role in the detection of anatomical abnormalities of the retina. It is based on the principle of low-coherence interferometry, where echo time delay and the magnitude of light reflected from the tissue is measured. Spectral domain OCT allows visualization of high resolution cross-sectional retinal images at different depths and disruptions across retinal layer interfaces (11).

In the analysis and interpretation of OCT images, it is most common to look at total retinal thickness, but a study by the Diabetic Retinopathy Clinical Research Network has shown only a moderate relationship between central retinal thickness and visual acuity (VA) (12). The changes in VA may be due other factors such as selective retinal layer changes or disruptions between layers.

OCT also allows quantitative measurement and mapping of the individual layers that comprise the retina. Previous OCT studies have shown that individual retinal layers are differentially affected by DR. Specifically, studies have reported thickening of the INL and OPL layers in subjects with diabetic macular edema (DME) and thinning of NFL and GCLIPL layers in subjects with minimal or no DR (6; 13; 14; 15; 16; 17) . Moreover, VA has been correlated with

thickness measures of the combined ganglion cell and inner plexiform layers (GCLIPL) and photoreceptor outer segment layer (OSL) (7; 18).

In addition to retinal thickness measurements, retinal reflectance has also been evaluated using OCT. Reflectance is a measure of the amount of light reflected from a given retinal layer. Regions with high intensity may have weak reflectivity whereas regions with low intensity may have strong reflectivity. Further, based on the hyper-reflectivity (dark-to-bright or bright-to-dark transitions) of the layers in an OCT scan, alterations in the integrity of the inner segment ellipsoid layer, have been reported (19; 20). Alterations in reflectance with respect to the INL and OSL layers in DR subjects have also been reported (21; 22).

A third measure that can be derived by OCT is interface disruption. This is a region where two layers cannot be visually distinguished or are disorganized. Studies have shown that disruptions of the photoreceptor layers and external limiting membrane are associated with reduced VA (23; 24; 25; 26).

En face imaging has emerged as a technique that permits enhanced visualization of retinal structural anomalies in two dimensions. Methods for generating en face thickness maps and reflectance images have been reported previously and have shown alterations at different stages of DR (21; 27; 28). In DR subjects, en face reflectivity images have shown reduced intensity regions due to edema in the inner and outer segment layers, indicative of photoreceptor loss (22). In addition, a study has demonstrated that pathological changes, like

intra-retinal fluid and hard exudates are indicated through the alterations in the en face retinal layer reflectance (29).

Although individual studies have greatly increased our understanding of the effects of DR on retinal thickness, reflectance, and disorganization, no study to date has derived these measures simultaneously from the same sample of diabetic patients. Consequently, it is not clear how these metrics relate to each other and their relationships to VA are also unclear. Moreover, the images used in some of the previous studies were obtained from low-density OCT B-scans. In the current research study, anatomical changes (thickness, reflectance, disorganization) in each retinal layer were assessed across different stages of DR, which permits examination of the multi-factorial changes responsible for reduced VA in DR.

1.4 RESEARCH OBJECTIVES

The current research study aims at accomplishing two main objectives, first to establish the correlation between VA and structural measures including retinal layer thickness, reflectance, and interface disruption. Second, examine alterations in retinal layer thickness and reflectance over time in subjects classified by treatment. Hence, the study may provide better insights about retinal layer alterations, which can be useful for monitoring the progression of DR, thereby contributing to the prevention of vision loss due to DR.

CHAPTER 2

METHODS

2.1 SUBJECTS

Approval for the research study was obtained from an Institutional Review Board at the University of Illinois at Chicago. The study was explained to all the subjects and informed assent was acquired, before enrollment, in adherence with the tenets of the Declaration of Helsinki. A total of 149 diabetic subjects participated in the study. Data regarding age, gender, race, duration of diabetes, HbA1c and number of anti-VEGF injections received were collected from the subjects. All the subjects underwent dilated fundus examination. In the cross-sectional study, based on clinical diagnosis 149 diabetic subjects were categorized into no DR (NDR; N=50), non-proliferative DR (NPDR; N=59) and proliferative DR (PDR; N=40). In the longitudinal study, twenty-three subjects of the 149 diabetic subjects returned for follow-up. Mean duration between baseline and follow-up was 203 ± 142 days. The subjects in the longitudinal study were classified into three groups based on whether the subject received anti-VEGF treatment or not. Subjects who never received treatment were categorized into

never treated group (NT; N=6), subjects who received treatment prior to baseline, but not between visits, were grouped as untreated (UT; N=7) and subjects who received treatment between baseline and follow-up were designated as treated (T; N=10). Exclusion criteria were clinical diagnosis of glaucoma, age-related macular degeneration, refractive error greater than 6 diopters of myopia, retinal vascular occlusions or any other conditions that could alter anatomic integrity of retina, history of intraocular surgery, cataract surgery performed less than 4 months prior to imaging, lens nuclear sclerosis score greater than 2+ or posterior sub-capsular cataract concurrent with VA less than 20/20. One eye per subject was selected based on the criteria, if both the eyes qualified, the eye with better image quality was included.

2.2 IMAGE ACQUISITION

For the current study, Spectral domain OCT (SDOCT) retinal imaging was performed using a commercially available instrument (Spectralis; Heidelberg Engineering, Heidelberg, Germany), over a retinal area of 20^{o} X 15^{o} centered on the fovea. The high density SDOCT raster volume scan of the macula consisted of 73 horizontal B-scans, averaged from 9 frames, with vertical spacing of 62 μm spacing between scans. B-scans were composed of 1024 A-scans and a depth resolution of 3.9 μm.

2.3 IMAGE ANALYSIS

2.3.1 Identification of retinal cell layers:

The acquired SDOCT images were processed and analyzed using our previously described semi-automated image segmentation software (21). The software identified eight interfaces between retinal cell layers in the SDOCT B-scans. It was developed in MATLAB (MathWorks, Inc., Natick, MA, USA) using graph theory and dynamic programming. As per the graph theory, a graph was created for each SDOCT scan, based on vertical gradients in the image, edge weights of the graph were assigned in a way so that the large gradients resulted in small weights. Using Dijkstra's algorithm, a horizontal path through the graph minimizing the total sum of weights was found, which defined a line separating two retinal cell layers. Depending on the sign of the gradients (positive or negative), weights of the graph were assigned and retinal cell layers with bright to dark transition or dark to bright transition were identified.

Figure 1 illustrates the segmented retinal layer interfaces. The following eight retinal cell interfaces were identified, the vitreous and nerve fiber layer (NFL), the NFL and combined ganglion/inner plexiform layers (GCLIPL), the GCLIPL and inner nuclear layer (INL), the INL and outer plexiform layer (OPL), the OPL and photoreceptor outer segment layer (OSL), the OSL and retinal pigment epithelium (RPE) and the RPE and choroid. The segmentation was carried out in eight consecutive steps, so that each retinal cell interface was given a unique path. The interface between vitreous and NFL had markedly largest dark to bright transition

(large positive vertical gradient) in the image and represented the lowest weighted path in the entire graph, hence, this interface was identified in the first step. In the next step, the search area in the graph was confined to include only image regions external to vitreous/NFL interface and then the OSL/ONL interface was identified. In the third step, to determine RPE/choroid interface the graph search area was restricted to include only regions external to ONL/OSL path and lower graph weights were assigned to larger negative gradients, thus marking a bright to dark transition. Fourth, by limiting the graph to encompass only image regions between the vitreous/NFL and ONL/OSL paths, the INL/OPL cell interface was detected. In fifth step, the OPL/ONL cell interface was traced, this was carried out by narrowing down the search area to include only the regions between the INL/OPL and ONL/OSL paths and allocating lower weights of the graph for higher negative gradients i.e., bright to dark transition. Sixth, the GCLIPL/INL cell interface was identified by restricting the graph search area to regions which are within 20-pixel proximity (internal) to the INL/OPL cell interface and hence seeking for a bright to dark transition. Seventh, to detect bright to dark transition the graph search area was limited to include only regions of the image between the vitreous/NFL and GCLIPL/INL cell interfaces and thus, the NFL/GCLIPL cell interface was recognized. In the final step, the OSL and RPE cell interface was detected by confining the graph search area to include only image regions between the previously identified ONL/OSL and RPE/choroid interfaces and recognizing a dark to bright transition.

Following the segmentation of the retinal cell interfaces, the software permitted an operator to review all the 73 segmented B-scans. In case of error in the segmentation, primarily due to pathological abnormalities, the operator could manually correct the errors in the detected surfaces. This was done by selecting the line to be corrected and drawing a revised line based on visual perception of the cell layer interface. The code then regenerated an automated line by limiting the graph search area to a small vertical region of the image surrounding the manually drawn line and revaluating the minimum graph cut solution. In addition, the program permitted identification of locations of disrupted regions, that is, the areas of cell interfaces between two retinal layers that are visually indiscernible. This was carried out manually by an operator.

2.3.2 Generation of en face thickness maps and reflectance images:

En face imaging is a technique which combines SDOCT scans and produces a frontal view of the retinal layers. It is a useful method to quantify the spatial extent of alterations due to DR progression and treatment. Previously, methods have been reported for the generation of en face thickness maps and reflectance images for individual retinal layers using SDOCT scans (22; 23). After the retinal cell layers were segmented, en face thickness maps and reflectance images were generated for all seven retinal layers (NFL, GCLIPL, INL, OPL, ONL, OSL and RPE) based on the segmentation of the 8 retinal interfaces. The thickness of each retinal layer was calculated as the depth separation between the adjacent retinal cell interfaces. For example,

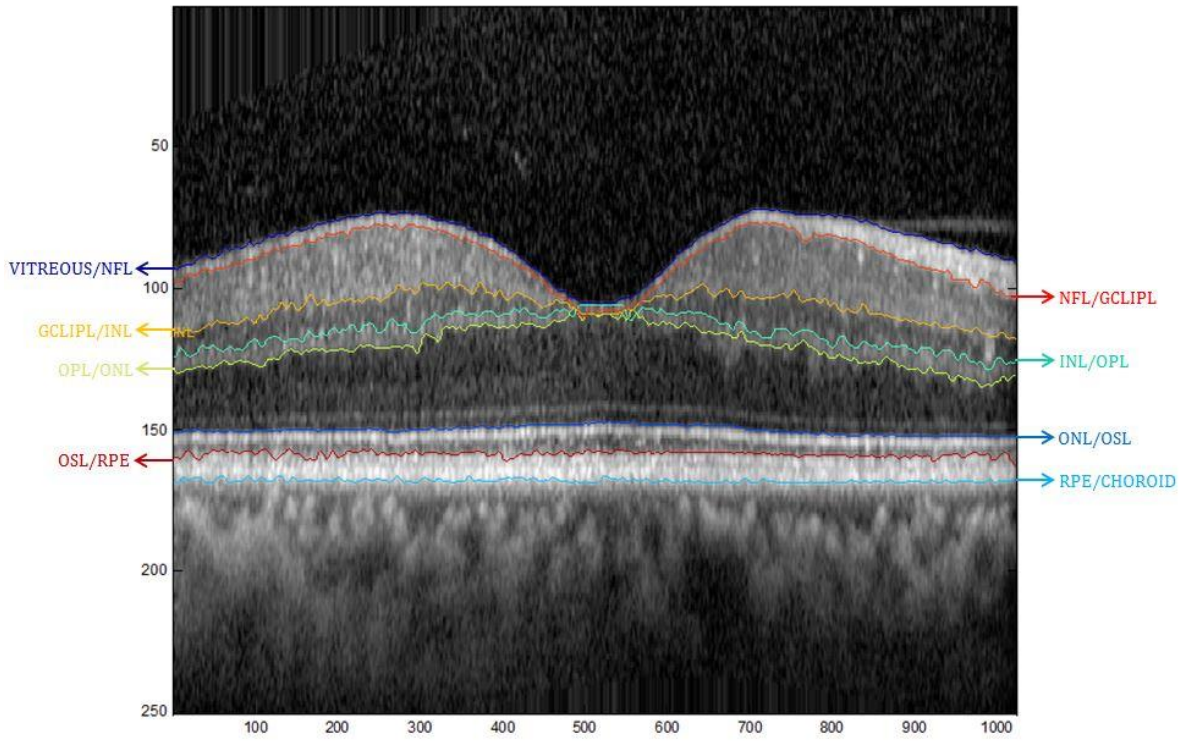

Figure 1: OCT B-scan of a mild NPDR subject with segmented retinal layers.

NFL thickness was estimated based on the depth separation between vitreous/NFL and NFL/GCLIPL cell interfaces. En face reflectance images were generated for each of the seven layers, by averaging the pixel values vertically within the segmented layers of each SDOCT B-scan.

Figure 2 displays an example of en face thickness maps and reflectance images of a mild NPDR subject (absence of disruption), corresponding to figure 1. Top (left to right): Central subfield thickness maps of NFL, GCLIPL, INL, OPL, ONL, OSL and RPE retinal layers. Bottom(left to right): Reflectance images of NFL, GCLIPL, INL, OPL, ONL, OSL and RPE retinal layers. The scale bars on the right side of each thickness map indicates the thickness measures of the layers in μm. The differences between reflectance images can be identified by comparing the brightness of each image. The gray scale ranges between 0 and 255.

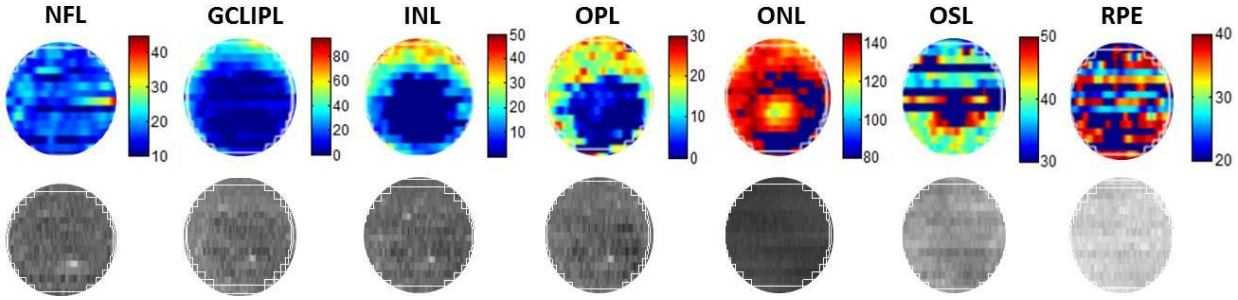

Figure 2: En face thickness maps and reflectance images in mild NPDR subject (without interface disruptions).

2.3.3 Quantification of retinal metrics:

Mean thickness values were measured for each retinal layer (NFL_T, $GCLIPL_T$, INL_T, OPL_T, ONL_T, OSL_T and RPE_T) in the ETDRS central subfield (1mm diameter) (30). The analysis was performed only on the central subfield because it is this region of the retina that is critical for visual acuity. Reflectance ratio values were also calculated in the central subfield for each layer. These metrics (NFL_R, $GCLIPL_R$, INL_R, OPL_R, ONL_R and OSL_R) were normalized with respect to the RPE layer, that is, the mean intensity of each layer divided by the mean intensity of the RPE ($Layer_{Intensity}/RPE_{Intensity}$).

Interface disruption areas (NFL_d, INL_d, ONL_d and RPE_d) were calculated relative to the total area in the ETDRS central subfield. NFL_d included interface disruption areas of both vitreous/NFL and NFL/GCLIPL interfaces, relative to the total area. Similarly, INL_d included interface disruption of both GCLIPL/INL and INL/OPL interfaces, ONL_d included interface disruption areas of both OPL/ONL and ONL/OSL interfaces and RPE_d included both disruptions of OSL/RPE and RPE/choroid interfaces. The regions with retinal layer interface disruption were not assigned any thickness or reflectance values.

Figure 3 illustrates an example of en face thickness maps and reflectance images in an NPDR subject with retinal layer interface disruptions. Top (left to right): Central subfield thickness maps of NFL, GCLIPL, INL, OPL, ONL, OSL and RPE retinal layers. Black regions are the areas with disrupted interfaces in which thickness and reflectance values were not assigned.

Bottom(left to right): Reflectance images of NFL, GCLIPL, INL, OPL, ONL, OSL and RPE retinal layers with disrupted interface areas shown in yellow. Major disruptions are identified at INL and OPL layer interfaces.

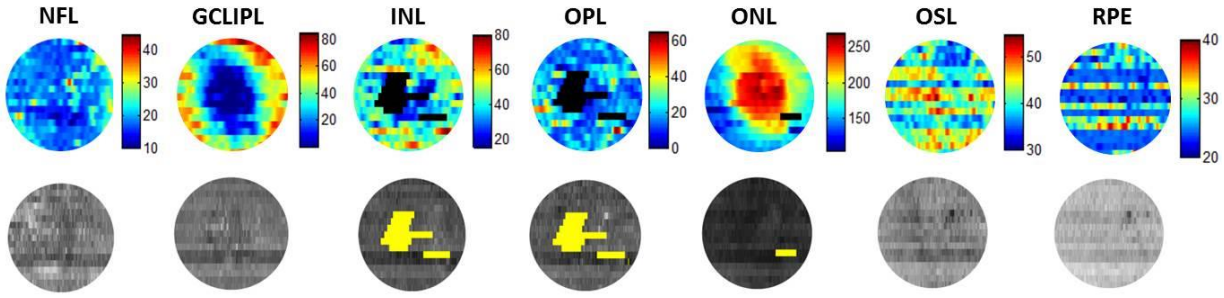

Figure 3: En face thickness maps and reflectance images in an NPDR subject, with interface disruptions

2.3.4 Clinical Measures

Visual acuity and retinal central sub-field thickness (CST) were recorded for all the subjects both at baseline and follow-up. Visual acuity was measured as the number of letters read at a 4-meter distance from the retro-illuminated Early Treatment Diabetic Retinopathy Study (ETDRS) charts by trained ophthalmic technician. The number of letters read was converted to log MAR (minimum angle of resolution) for analysis. Log MAR was obtained by

multiplying number of letters read by 0.02 and subtracting the product value from 1.1 (log MAR VA=1.1-(0.02*number of letters)). Where, 0.02 log units is the score value for each letter on the ETDRS chart. Retinal central sub-field thickness (CST) was measured using the Heidelberg Spectralis OCT software (Heidelberg Engineering, Heidelberg, Germany).

2.4 STATISTICAL ANALYSIS

2.4.1 Cross-sectional study:

One-way analysis of variance was used to compare age, diabetes duration, HbA1c levels among the three groups. A two-way repeated measures ANOVA with subject group (no DR, NPDR, PDR) and layer (NFL_T, $GCLIPL_T$, INL_T, OPL_T, ONL_T, OSL_T, RPE_T) as main effects was performed to compare retinal thickness values. Similarly, two-way repeated measures ANOVA with subject group and layer (NFL_R, $GCLIPL_R$, INL_R, OPL_R, ONL_R, OSL_R) as main effects were performed to compare retinal reflectance ratios. VA (log MAR) was compared among the three groups using a one-way ANOVA. To evaluate the relationship between VA and retinal thickness, as well as the relationship between VA and retinal reflectance, Spearman's Rank order correlation was performed. Pearson's correlation was not used for analysis, as the assumption of data being normally distributed was not satisfied. For the analysis of the areas of interface disruption, subjects were categorized into two groups based on the presence or

absence of interface disruptions and VA was compared between these groups using Mann-Whitney U test. Significance level was accepted at $P \leq 0.05$.

2.4.2 Longitudinal study:

Age, diabetes duration, HbA1c levels and time interval between visits were compared among the NT, UT and T groups using one-way analysis of variance (ANOVA). Two-way repeated measures ANOVA with subject group (NT, UT, T) and visit (baseline and follow-up) as main effects were performed to compare retinal thickness values at the two visits. Similarly, a two-way repeated measures ANOVA with subject group and visit as main effects was performed to compare retinal reflectance values at the two visits. To identify differences between groups at the two visits, Bonferroni-corrected post-hoc comparisons were performed

CHAPTER 3

RESULTS

3.1 CROSS-SECTIONAL STUDY: CORRELATION OF RETINAL LAYER METRICS WITH VA

3.1.1 Demographics of all DR subjects:

The mean age of the DR subjects was 56 ± 12 years (N=149). A one-way ANOVA that assessed age differences within the DR subgroups indicated that the mean age of the subjects in the NPDR (59 ± 11 years) and PDR (53 ± 11 years) groups did not differ significantly from the no DR group (55 ± 13 years) (P=0.11 and P=0.35, respectively). However, the mean age of the subjects differed between NPDR and PDR groups (P<0.01). The mean duration of diabetes differed significantly among the no DR (15 ± 16 years), NPDR (23 ± 19 years), and PDR groups (26 ± 18 years) groups (P<0.01). Mean duration of diabetes was similar between NPDR and PDR groups (P=0.46), but was significantly different between the NPDR and PDR groups when compared to no DR group (P<0.05). Mean HbA1c was 7.67 ± 3.75%, 9.38 ± 3.24% and 8.76 ± 2.69% in no DR, NPDR and PDR groups, respectively (P=0.03). The mean HbA1c level was

higher in NPDR compared to the no DR group (P=0.01), but was similar between NDR and PDR groups (P=0.11) and between NPDR and PDR groups (P=0.30).

3.1.2 Variation of VA (log MAR) and CST among no DR, NPDR and PDR groups:

The mean log MAR VA of the DR subjects was 0.08 ± 0.18 (N=149). A one-way ANOVA indicated significant log MAR differences among the three groups ($F = 8.82$. $P < 0.001$). Pairwise comparisons among the three groups indicated significant differences between the no DR (0.00 log MAR) and NPDR (0.12 log MAR) groups ($t = 3.46$, $p = 0.002$). As well as significant differences between the no DR and PDR (0.14 log MAR) groups ($t = 3.77$, $P < 0.001$). The NPDR and PDR groups did not differ significantly ($t = 0.66$, $P = 1$).

Mean CST, which was obtained from the Heidelberg Spectralis OCT software, was 284 ± 56 μm for the complete sample of subjects (N=149). CST values for the individual groups are provided in Table 1. A one-way ANOVA was used to compare CST among the three groups. Mean CST was significantly different among no DR, NPDR and PDR groups (F=4.67; P=0.01). When compared to the NDR group, CST was significantly higher in the NPDR (t=1.99; P<0.01) and PDR groups (t=2.01; P=0.02), but not significantly different between NPDR and PDR groups (t=2.00; P=0.80).

For all subjects, CST was correlated with VA (ρ=0.21; P=0.01), such that subjects with retinal thickening (increased CST) had reduced VA. However, CST was not significantly correlated with VA within any of the subgroups (all $\rho \leq 0.26$; $P \geq 0.09$).

3.1.3 Individual retinal layer thickness and association with VA:

The mean retinal thickness measurements derived using the automated segmentation algorithm for each subject group are provided in Table 1. A two-way repeated measures ANOVA was performed with group and layer as main effects. The ANOVA indicated a significant effect of layer ($F = 1.59$, $P < 0.001$), but not group ($F = 2.44$, $P = 0.09$). The interaction between group and layer was significant ($F = 4.06$, $P < 0.001$). Bonferroni-corrected pairwise comparisons were performed, which indicated that the groups did not differ significantly for any retinal layer.

Spearman's correlation coefficients (ρ) were calculated to evaluate the relationship between the individual retinal layer thickness and log MAR VA, as well as the relationship between CST and VA (Table 1). Increased INL_T was associated with reduced VA, ($\rho=0.47$; $P<0.01$; $N=149$). Further, increased INL_T was correlated with reduced VA in the NPDR and PDR groups (both $\rho\geq0.34$; $P<0.01$), but not with the no DR group ($\rho=0.25$; $P=0.08$). OPL_T was correlated with VA, such that subjects with reduced VA had increased OPL_T ($\rho=0.24$; $P=0.003$; $N=149$). However, OPL_T was not significantly correlated with VA for any of the groups individually (all $\rho\leq0.21$; $P\geq0.20$). Although RPE_T was not associated with VA for the complete sample of DR subjects, there was a significant correlation between VA and RPE_T in the PDR group, such that subjects with greater RPE_T had reduced VA ($\rho=0.32$; $P=0.04$).

Table 1: Mean thickness measures of individual retinal layer and Spearman's correlation coefficients with VA in all DR subjects (N=149)

RETINAL LAYER	GROUP	THICKNESS (μm) (Mean ± SD)	ρ
NFL	No DR	18 ± 3	0.08
	NPDR	19 ± 5	-0.05
	PDR	19 ± 4	0.22
	All subjects	19 ± 4	0.08
GCLIPL	No DR	31 ± 13	0.04
	NPDR	30 ± 15	0.07
	PDR	32 ± 23	0.02
	All subjects	31 ± 17	0.005
INL	No DR	18 ± 6	0.25
	NPDR	26 ± 18	0.34**
	PDR	41 ± 48	0.55**
	All subjects	28 ± 29	0.47**
OPL	No DR	17 ± 6	0.15
	NPDR	20 ± 11	0.16
	PDR	21 ± 7	0.21
	All subjects	19 ± 8	0.24**
ONL	No DR	113 ± 12	-0.06
	NPDR	132 ± 44	0.20
	PDR	118 ± 34	-0.02
	All subjects	122 ± 34	0.12
OSL	No DR	45 ± 7	0.05
	NPDR	43 ± 7	-0.17
	PDR	43 ± 9	-0.17
	All subjects	41 ± 8	-0.14
RPE	No DR	28 ± 6	-0.13
	NPDR	27 ± 5	-0.16
	PDR	25 ± 4	0.32*
	All subjects	27 ± 5	-0.12
CST	No DR	264 ± 24	0.05
	NPDR	292 ± 55	0.22

	PDR	296 ± 78	0.26
	All subjects	284 ± 56	0.21**

* indicates P≤0.05; ** indicates P ≤ 0.01

3.1.4 Individual retinal layer reflectance and association with VA:

Table 2 provides the mean reflectance values for the individual retinal layers (NFL_R, $GCLIPL_R$, INL_R, OPL_R, ONL_R, OSL_R) for the various subject groups. A two-way repeated measures ANOVA was performed with group and layer as main effects. The ANOVA indicated a significant effect of layer ($F = 1959.76$, $p < 0.001$), but not group ($F = 2.02$, $p = 0.13$). The interaction between group and layer was significant ($F = 3.01$, $p < 0.001$). Bonferroni-corrected pairwise comparisons were performed, which indicated that the groups did not differ significantly for any retinal layer.

Spearman's correlation coefficients were calculated to evaluate the relationship between the individual retinal layer reflectance ratio and log MAR VA. Table 2 provides the Spearman's correlation coefficients that show the relationship between VA retinal reflectance ratio. Increased NFL_R was correlated with reduced VA for the complete sample of DR subjects ($\rho=0.24$; $P=0.003$; $N=149$). Further, high NFL_R was also associated with reduced VA in the NPDR group ($\rho=0.28$; $P=0.03$), but no significant correlations were observed for the no DR and

Table 2: Mean reflectance measures of individual retinal layer and Spearman's correlation coefficients with VA in all DR subjects (N=149)

RETINAL LAYER	GROUP	REFLECTANCE RATIO (AU) (Mean ± SD)	ρ
NFL	No DR	0.53 ± 0.07	0.18
	NPDR	0.57 ± 0.08	0.28
	PDR	0.57 ± 0.11	0.09
	All subjects	0.56 ± 0.09	0.24**
GCLIPL	No DR	0.61 ± 0.06	0.08
	NPDR	0.62 ± 0.07	0.23
	PDR	0.61 ± 0.08	0.002
	All subjects	0.61 ± 0.07	0.12
INL	No DR	0.50 ± 0.05	0.02
	NPDR	0.51 ± 0.06	-0.05
	PDR	0.49 ± 0.08	-0.21
	All subjects	0.50 ± 0.06	-0.09
OPL	No DR	0.52 ± 0.06	0.05
	NPDR	0.52 ± 0.06	-0.05
	PDR	0.51 ± 0.08	-0.02
	All subjects	0.52 ± 0.07	-0.05
ONL	No DR	0.36 ± 0.04	0.04
	NPDR	0.37 ± 0.04	-0.05
	PDR	0.38 ± 0.06	0.06
	All subjects	0.37 ± 0.05	0.07
OSL	No DR	0.81 ± 0.06	-0.09
	NPDR	0.79 ± 0.08	-0.27*
	PDR	0.79 ± 0.10	-0.46**
	All subjects	0.80 ± 0.08	-0.29**

* indicates P≤0.05; ** indicates P ≤ 0.01

PDR groups (both $\rho \leq 0.22$; $P \geq 0.17$). Additionally, low OSL_R was associated with reduced VA for the complete sample of subjects ($\rho = -0.29$; $P < 0.01$; $N = 149$). When compared within the subgroups, low OSL_R was significantly correlated with reduced VA in the NPDR and PDR groups (both $\rho \leq -0.27$; $P < 0.05$), but not in the no DR group ($\rho = -0.09$; $P = 0.56$). No other reflectance ratios were significantly associated with VA.

3.1.5 Comparison of VA in subjects with and without retinal layer disruptions:

For DR subjects who had layer interface disruptions, the mean percentage of the foveal region that was disrupted in the inner nuclear layer (INL_d) and outer nuclear layer (ONL_d)was 27 ± 31% and 28 ± 33%, respectively. There were no disruptions in the NFL/GCLIPL and OSL/RPE interfaces. Mean log MAR for subjects who had disruptions in the INL was 0.16 ± 0.17 (N=25), whereas the mean log MAR for subjects who did not have disruptions was 0.07 ± 0.18 (N=124); VA was lower in subjects with the presence of INL_d (P=0.003). Mean log MAR for subjects who had disruptions in the ONL was 0.17 ± 0.16 (N=26), whereas the mean log MAR for subjects who did not have disruptions was 0.07 ± 0.18 (N=123); VA was lower in subjects with the presence of ONL_d (P=0.001).

3.2 CHANGES IN THE RETINAL LAYER METRICS OVER TIME

3.2.1 Demographics of subjects in NT, UT and T groups:

Table 3 represents the demographic details of the subjects in the NT, UT and T groups. All the comparisons were performed using one-way ANOVA. There were no significant differences in subject age among the groups. All the subjects had similar mean HbA1c levels and diabetes duration did not vary among the three groups. The mean follow-up duration was not significantly different among the groups; hence this factor was not considered further.

Table 3: Demographics of the subjects in NT, UT and T groups

PARAMETER	NT (N=6) (Mean ± SD)	UT (N=7) (Mean ± SD)	T (N=10) (Mean ± SD)	P-VALUE
Age (years)	53 ± 15	59 ± 10	55 ± 7	0.55
Diabetes duration (years)	19 ± 7	19 ± 9	20 ± 8	0.96
Follow-up duration (days)	230 ± 155	154 ± 73	220 ± 172	0.57
HbA1c (%)	8.3 ± 1.1	9.3 ± 1.5	8.6 ± 1.8	0.52
Injections in Lifetime (before baseline and between visits)	0	3 ± 2	9 ± 5	0.006

3.2.2 Variations in retinal metrics between visits and among NT, UT and T groups:

A two-way repeated measures ANOVA was performed to compare the mean log MAR values at the two visits for the NT, UT and T groups. The ANOVA showed a significant effect of group ($F = 3.64$, $P = 0.05$), but not visit (mean log MAR was 0.05 at visit-1 and 0.06 at visit-2; $F = 0.06$, $P = 0.80$). The group by visit interaction was not significant ($F = 0.77$, $P = 0.48$). Mean log MAR was significantly lower in UT compared to T group ($t = 2.67$, $P = 0.048$), but there were no significant differences among other groups (all $t < 1.89$, $p > 0.23$).

A series of two-way repeated measures ANOVA with group (NT, UT and T) and visit as main effects was performed to evaluate potential changes in retinal thickness between visits. ANOVA identified significant changes between visits in retinal thickness of two layers: RPE_T and OSL_T. For the RPE, there was a significant difference between visits for the complete sample of subjects (visit-1 = 25μm, visit-2 = 28 μm; $F = 5.51$, $P = 0.03$), but the three groups had similar RPE_T (NT = 27 μm; UT = 26 μm; T= 26 μm; $F = 0.47$, $P = 0.64$). There was no significant interaction ($F = 0.00$, $P = 1.0$). There was also a significant difference between visits in OSL_T for the complete sample of subjects (visit1 = 24.89 μm, visit-2 = 28.34 μm $F = 7.41$, $P = 0.01$), but the three groups had similar OSL_T (NT = 27.84 μm; UT = 25.61 μm; T = 26.39 μm; $F = 1.19$, $P = 0.63$). There was no significant interaction ($F = 1.52$, $P = 0.25$). No other statistically significant differences were observed.

A series of two-way repeated measures ANOVA with group (NT, UT and T) and visit as main effects was also performed to evaluate changes in retinal reflectance between visits. No significant differences were observed between visits ($F \leq 0.81$, $P \geq 0.31$). For comparisons among the groups, only the INL_R was significantly different. In comparison to the NT group (reflectance = 0.54), the INL_R was lower in the UT group (reflectance = 0.45; $t=2.10$, $P=0.005$), but there were no significant differences among other groups, NT/T and UT/T (Reflectance =0.51; $t=2.05$, $P>0.05$). The interaction was not significant ($F \leq 2.77$, $P \geq 0.09$).

CHAPTER 4

DISCUSSION

In the current thesis, alterations in individual retinal layer metrics (thickness, reflectance and interface disruptions) were concurrently determined by en face OCT imaging in subjects at different stages of DR and the association of these metrics with VA was determined. Assessment of retinal layer interface disruption and reflectance provide important complementary metrics to thickness, which is the standard measure derived from OCT images. In addition, changes in the three metrics (thickness, reflectance, disruption) were examined over time in subjects grouped by treatment; despite the small sample size, this is the first report of concurrent measurement of these values over time.

4.1 IMAGE SEGMENTATION METHODS AND ANALYSIS

Methods for segmentation of retinal layers in DR have been reported in quite a few previous studies (21; 31; 32; 33; 34; 35; 36). Segmentation of the retina is important, as the effects of disease, such as DR, may have selective effects on individual retinal layers. Implementation of en face OCT imaging for analyzing retinal layer alterations in DR is particularly advantageous,

as it allows visualization of spatially localized thickness and reflectance abnormalities. In general, the presence of distinct interfaces between layers allows accurate automated segmentation of retinal layers in healthy individuals, as well as in most DR subjects. However, in some patients with advanced pathologies, the interfaces are not clearly identifiable even by an expert human observer. To assess the retinal integrity, the current study presents an interface disruption metric, a quantitative measure of the regions between layers that are visually indiscernible due to pathologies and macular edema.

4.2 ASSOCIATIONS OF VA WITH RETINAL LAYER METRICS

One important consideration in the decision of whether to initiate treatment in DR is whether the structural changes due to the disease affect retinal function or not. That is, treatment may not necessarily be warranted for individuals who have retinal structural abnormalities but no functional loss. Thus, determining the relationship between structural metrics and VA, the most common functional measure, is essential. The results of the current study showed that NFL_T, INL_T, OSL_T, NFL_R and OSL_R were the measures significantly associated with reduced VA.

The observed correlation of reduced VA with increased INL_T and OPL_T in DR subjects was consistent with previous studies that reported a correlation between increased edema and reduced VA (23). Here too, we attribute the thickening of these retinal layers and the associated VA loss to edema. The significant correlation between INL_T and VA within the NPDR

and PDR groups suggests that assessment of this layer may be useful as a metric for assessing visual outcome in future clinical trials. The findings of the study are also in agreement with other studies that reported the presence of cystoid spaces and increased INL and OPL thickness, although these previous studies did not report correlations with VA (5; 6). The association of CST, measured from Heidelberg software, with reduced VA was also consistent with previous studies (37; 12).

Although reflectance is not a common measure derived from OCT images, the current study reports the association of decreased OSL_R with reduced VA. This finding agrees with a previous study that reported reduced photoreceptor reflectance due to the presence of cystoid spaces in OSL (38). It is also consistent with studies that demonstrated the association of the integrity of the photoreceptor layer with VA (20; 23; 24; 31; 39). However, these previous studies assessed inner and outer receptor interface visibility based on single or low density raster volume of B-scans, whereas the current study used en face reflectance images generated from high density OCT B-scans. Thus, the present study provides a more accurate evaluation of localized OSL deficits compared to the evaluation of individual B-scans. In addition, the present study also reports a significant correlation between increased NFL_R and reduced VA, which has not been reported previously in DR.

In subjects with who had interface disruptions in the INL and ONL, VA was reduced, relative to individuals who did not have disruption in these layers. This finding is in consensus with

previous studies that reported a correlation between combined inner retinal interface disruption (NFL/GCLIPL, GCLIPL/INL/OPL, OPL/ONL) and VA, although they did not assess the disruptions in other layer interfaces (ONL/OSL, OSL/RPE) (25; 26).

4.3 CHANGES IN RETINAL METRICS AMONG NT, UT AND T GROUPS OVER TIME

Amongst the quantitative metrics analyzed, as anticipated, there were no significant differences in the never treated group over time, as the subjects were naïve to anti-VEGF treatment. Although RPE_T was significantly different between baseline and follow-up, averaged across NT, UT and T groups, the mean thickness increase was only of 2.21 μm. The probable reason for no significant alterations in other parameters could be an effect of the treatment received prior to baseline, resulting in stability of disease progression. OSL_T increased from baseline to follow-up, averaged across three groups. Moreover, a lower reflectance ratio of the INL layer was observed in the UT group. The alterations may be indicative of the additional treatment required to prevent further progression.

4.4 LIMITATIONS AND FUTURE WORK

Variability in the treatment protocol among the subjects and small sample size limited detection of statistically significant differences in the follow-up study that examined the

retinal metrics over time. Future studies with a larger sample size and grouping of subjects based on number of anti-VEGF injections may be helpful in reducing the variability. Subjects with advanced DR have pathologies which cause severe abnormalities in the retinal layer integrity and this may have limited our ability to accurately segment the retinal layers. Although errors in the segmentation were corrected manually, subjective errors were avoided by repeating the segmentation using the same graph theory, but applied to a confined region. Quantifying the interface disruption, however, requires manual recognition and labeling of the disrupted regions, which is time consuming and can be subjective. To improve the efficacy of the method, development of a technique that can automate the identification and mapping of the retinal layer interface disruptions is required.

Although subjects with significant cataract were excluded from the study, inclusion of subjects with some degree of cataract may have affected VA, which is another limitation. However, since DR is known to cause lens opacities, excluding subjects with any degree of cataract would have significantly reduced the sample size. Future studies, with larger sample size are required, that can account for potential confounding factors responsible for loss of VA, substantiate the current findings, and reveal differences that may not have been apparent with the current sample size.

4.5 CONCLUSION

Although enface imaging has been performed in the past, comprehensive and combined assessment of individual retinal layer thickness, reflectance, and interface disruptions, as well as their association with visual acuity, have not been reported. Clinical treatment based on specific retinal layer metrics, rather than total retinal thickness, may potentially provide better outcomes to prevent vision loss. The results of the current thesis showed that alterations in retinal metrics in individual retinal layers were associated with reduced VA. Assessment of these select layers may be helpful in monitoring visual outcomes due to progression of DR. Similarly, the thickness changes that were observed in subjects who received treatment may be of use in stipulating further therapeutic intervent

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APPENDIX

APPROVAL FROM INSTITUIONAL REVIEW BOARD

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

October 12, 2016

Mahnaz Shahidi, PhD
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RE: Protocol # 2013-1168
"Ocular Biomarkers in Diabetes"

Dear Dr. Shahidi:

Your Continuing Review was reviewed and approved by the Expedited review process on October 12, 2016. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: October 19, 2016 - October 19, 2017
Approved Subject Enrollment #: 350
Additional Determinations for Research Involving Minors: The Board determined that this research satisfies 45CFR46.404, research not involving greater than minimal risk. Therefore, in accordance with 45CFR46.408, the IRB determined that only one parent's/legal guardian's permission/signature is needed. Wards of the State may not be enrolled unless the IRB grants specific approval and assures inclusion of additional protections in the research required under 45CFR46.409. If you wish to enroll Wards of the State contact OPRS and refer to the tip sheet.
Performance Sites: UIC
Sponsor: National Institute of Diabetes and Digestive & Kidney Disease
PAF#: 00003539
Grant/Contract No: DP3 NK104393
Grant/Contract Title: Ocular Biomarkers of Microvascular, Neural and Metabolic Function in Diabetes
Research Protocol:

a) Ocular Biomarkers in Diabetes; Version 1.3, 09/23/2014

Recruitment Materials:

a) Adult Flyer, Version 1.0, 12/08/2014
b) Child Flyer, Version 1.0, 12/08/2014

Informed Consents:

a) Ocular Biomarkers in Diabetes, Version 1.3, 09/23/2014
b) Waiver of informed consent granted [45 CFR 46.116(d)] for the identification of potential subjects in the recruitment phase of the research.

Assent:

a) Ocular Biomarkers in Diabetes, Version 1.1, 9/23/2014

HIPAA Authorizations:

a) Ocular Biomarkers in Diabetes, Version 1.3, 09/23/2014
b) Review Preparatory to Research acknowledged [45 CFR 164.512(i)(1)(ii)]

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific categories:

(4) Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving X-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.) Examples: (a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the subject or an invasion of the subject's privacy; (b) weighing or testing sensory acuity; (c) magnetic resonance imaging; (d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler blood flow, and echocardiography; (e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.,
(5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis).,
(6) Collection of data from voice, video, digital, or image recordings made for research purposes.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
09/26/2016	Continuing Review	Expedited	10/12/2016	Approved

Please remember to:

→ Use your **research protocol number** (2013-1168) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements as explained in the following, which are posted on

the OPRS website: *http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/index.shtml*):
"UIC Investigator Responsibilities, Protection of Human Research Subjects"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-2053. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Laura Litman
IRB Coordinator, IRB # 3
Office for the Protection of Research Subjects

Enclosures:

1. **Informed Consent Document:**
   a) Ocular Biomarkers in Diabetes, Version 1.3, 09/23/2014
2. **Assent Document:**
   a) Ocular Biomarkers in Diabetes, Version 1.1, 9/23/2014
3. **Recruiting Materials:**
   a) Adult Flyer, Version 1.0, 12/08/2014
   b) Child Flyer, Version 1.0, 12/08/2014

cc: Mark I. Rosenblatt, Ophthalmology and Visual Sciences, M/C 648
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