STRUCTURAL AND FUNCTIONAL CORRELATIONS IN DIABETIC RETINOPATHY

THESIS

Submitted in partial fulfillment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

by

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CERTIFICATE

This is to certify that the thesis entitled **"Structural and Functional Correlations in Diabetic Retinopathy"** submitted by Gella Laxmi, ID No 2009PHXF013G for the award of Ph.D. degree of the Institute, embodies original work done by her under my supervision.

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ABSTRACT

Aim: To elucidate the structural changes of retina using Spectral Domain Optical Coherence Tomography (SD-OCT), B-scan Ultrasonography and functional damage using Pelli-Robson contrast sensitivity chart, FM 100-hue test and microperimeter (MP1) among subjects with diabetes with and without diabetic retinopathy in a population based cohort.

Methods: Subjects with diabetes with and without DR from Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study (SN-DREAMS) were included in the current study. Subjects underwent comprehensive eye examination which included imaging tests: SD-OCT, B-scan ultrasonography and functional tests: Contrast sensitivity using Pelli-robson chart, FM-100 Hue colour vision test, Microperimetry.

Main outcome of this study was to assess the structural and functional changes of the retina among subjects with diabetes and to determine the relationship between those structural and functional changes in a population based sample.

Results: A total of 867 subjects with diabetes with various stages of DR were included for analysis. SD-OCT revealed significant difference in the retinal thickness between subjects with diabetes, with and without retinopathy only in the superior ($p= 0.044$) and temporal ($p= 0.011$) quadrants. Men had significantly greater CFT, CSFT, RNFL thickness and retinal thickness in all the quadrants of 3mm and 6mm zones compared to women ($p< 0.001$). On comparison of diabetic subjects with no DR and those with mild and moderate NPDR, CFT was significantly high and the RPE thickness was significantly low in mild and moderate NPDR (CFT: No DR - 167.28 ± 14.56 µm, Mild and moderate NPDR - 177.12 ± 20.99 µm; $p= 0.003$. RPE thickness: No DR - 43.25 ± 6.90 µm, Mild and moderate NPDR - 40.51 ± 6.14 µm; $p= 0.014$).

Photoreceptor layer thickness was significantly reduced in diabetic subjects with no DR compared to non-diabetic subjects (non-diabetic - 65.04 ± 4.43 μm, no DR - 62.75 ± 4.82 μm; p= 0.014).

Most frequently found lesions on SD-OCT are hard exudates, hemorrhages and cystic spaces. Four morphological patterns of hemorrhages noted on SD-OCT are oval shaped hyper reflective echo's surrounded by hypo reflectivity, medium to high reflective echo's causing shadow in the inner retina, high reflective echo's at RNFL and ganglion cell layer causing shadow in case of flame shaped and organized high reflective membrane at vitreoretinal interface causing shadow in case of subhyaloid hemorrhage. Hard exudates are located in the inner and outer nuclear and plexiform layers. Cystic spaces involve layers from outer nuclear layer to ganglion cell layer. Retinal thinning was noted in the areas of capillary non-perfusion. Neovascularization appear as a pre-retinal medium reflective membrane causing shadow of underlying layers.

Foveal slope profile in subjects with diabetes and DR was calculated using a mathematical model and report that the average foveal slope values among subjects with diabetes in superior, inferior, temporal, and nasal quadrants were 8.94 ± 3.04°, 8.71 ± 3.14°, 7.86 ± 2.60°, and 8.07 ± 2.72° respectively. Shallow foveal slope was found in subjects with STDR in inferior quadrant (STDR: 7.33 ± 6.26 vs controls: 10.31 ± 3.44; $p = 0.021$) when compared to non-diabetic subjects and in superior (STDR: 7.62 ± 5.81 vs No DR: 9.11 ± 2.82; $p = 0.033$), inferior (STDR: 7.33 ± 6.26 vs No DR: 8.81 ± 2.81; $p = 0.048$), and temporal quadrants (STDR: 6.69 ± 5.70 vs No DR: 7.97 ± 2.33; $p = 0.030$) when compared to subjects with no DR. Foveal slope was significantly correlated with age ($r = –0.241$; $p < 0.001$) and center subfield thickness ($r = –0.542$; $p < 0.001$). Dark and light pixel values from SD-OCT images were analyzed for subjects with diabetes without retinopathy and age matched controls. Dark pixel values were significantly high among

cases (Cases: 8488.68 ± 3094.70, Controls: 6794.85 ± 1877.83; p < 0.001). No significant difference was found in the light pixel values and the light-dark (LD) ratio between the groups.

Prevalence of impaired colour vision (ICV) among subjects with diabetes with no DR and those with DR was 42.7% (CI: 38.4- 47) and 43.4% (CI: 35.9 -50.9), respectively. Prevalence of ICV was significantly higher in men (54.4%) than in women (45.9%, *P* = 0.017). On multiple logistic regression analysis it was shown that women were at higher risk of developing ICV (OR: 1.43, (1.04-1.98)). Subjects with CSME were 3 times more likely to have ICV. STDR remained a significant risk factor for ICV (OR: 1.98, (1.05-3.73)). A history of cataract surgery was a protective factor (OR: 0.54, (0.34-0.86)). The most common ETDRS patterns of hue discrimination impairment in our study sample were C3 (28.7%), C2 (19%) and C1 (12.1%). Most of our subjects were found to have a blue-yellow colour defect. Among diabetic subjects with no retinopathy, significant risk factors for ICV were women (OR: 1.79, (1.00-3.18)), increased resting heart rate (OR: 1.04, (1.01-1.07)) and raised intraocular pressure (OR: 1.12, (1.00-1.24)).

Mean contrast sensitivity (CS) in our subjects with diabetes was 1.32 ± 0.20 log units, which was less than the 1.44 ± 0.05 log units that was reported earlier. Factors associated with reduced CS were increased age (β= - 0.007, *P* <0.001), increased duration of diabetes (β = -0.004, *P* = 0.002), increased vibration perception threshold score, i.e., diabetic neuropathy (β = -0.007, *P* <0.001), use of insulin (β = -0.06, *P* = 0.047), reduced hemoglobin (β= 0.012, *P* =0.002), poor best corrected visual acuity (BCVA) (β= -0.695, *P* <0.001), increased severity of DR (β = -0.029, *P*< 0.001), increased total error score on the FM 100-hue test (β = -0.001, *P* <0.001), reduced mean retinal sensitivity (β= 0.021, *P* <0.001), and presence of cataract. We developed a model to predict CS based on the systemic and ocular parameters.

Predicted CS = 1.699 + (- 0.563) Best corrected visual acuity + (- 0.003) Vibration perception threshold score + (-0.106) Presence of posterior subcapsular cataract + (- 0.002) Age + (- 0.016) Stage of DR.

Prevalence of abnormal mean retinal sensitivity (MRS) among the study sample was 89.1%. A significant decline in MRS was noted among subjects with diabetes but no retinopathy compared to healthy individuals. MRS was significantly reduced with increase in duration of diabetes (r= -0.139, p=0.008), increased HbA1c (r= -0.112, p=0.035), reduced BCVA (r= -0.366, p<0.001) and increased central foveal thickness (CFT) (r= -0.123, p= 0.025). On multivariate analysis increased CFT remained a single significant risk factor for abnormal MRS with OR 1.02. MRS was significantly reduced among subjects with altered foveal contour and abnormal inner retinal layers. Poor central and relatively unstable fixation were significantly associated with BCVA (p=0.002, p=0.017 respectively). Prevalence of scotoma was 24.4%, which was highly prevalent in women (p=0.035) and among subjects with reduced BCVA (p<0.001), reduced contrast sensitivity (p<0.001), cataract (p<0.001), impaired retinal sensitivity (p<0.001) and presence of STDR (p<0.001). Presence of scotoma was significantly associated with abnormal foveal contour (p=0.046) and altered inner retinal layers (p<0.001). Among those subjects with diabetes and poor central fixation, 80% did not have any DR, and, among those with relatively unstable fixation 78% did not have any DR.

Incidence of incomplete posterior vitreous detachment (IPVD) and complete posterior vitreous detachment (CPVD) from no PVD was 80.8% and progression to CPVD from IPVD was 32.63%. Subjects with CPVD and IPVD were older compared to those with no PVD. Factors associated with conversion of no PVD and IPVD to CPVD are increase in age (OR: 1.04 (1.01–1.06)) and increased axial length (OR: 1.34 (1.09–1.65)). Subjects with myopia are at 2.13 times

increased risk of developing CPVD (P = 0.009). Undergoing cataract surgery was associated with 2.4 times increased risk of developing CPVD (P = 0.031).

Conclusion: In this population based study we analyzed various structural and functional changes among subjects with diabetes with and without DR. Structural changes based on SD-OCT, we report early neuronal changes in terms of retinal thinning, thinning of photoreceptor layer, and increased dark pixel values among subjects with diabetes. We also report a trend of shallowing of foveal slope with increase in the severity of DR. Functional changes like contrast sensitivity, colour vision, retinal sensitivity and fixation characteristics were analyzed among the study sample. Various risk factors for the structural and functional changes were also analyzed.

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ABBREVIATIONS

BCVA	Best Corrected Visual Acuity
CS	Contrast Sensitivity
CC	Cortical Cataract
CSME	Clinically Significant Macular Edema
CFT	Central Foveal Thickness
CPVD	Complete Posterior Vitreous Detachment
CSFT	Central Subfield Thickness
CWS	Cotton Wool Spots
C-index	Confusion index
DM	Diabetes Mellitus
DR	Diabetic Retinopathy
DME	Diabetic Macular Edema
ETDRS	Early Treatment Diabetic Retinopathy Study
ELM	External Limiting Membrane
ERM	Epiretinal Membrane
ERG	Electroretinogram
FM 100	Farnsworth Munsell 100
FAZ	Foveal Avascular Zone
FA	Fluorescein Angiography
GCL	Ganglion Cell Layer
HbA1c	Glycosylated Hemoglobin
IDDM	Insulin Dependent Diabetes Mellitus
ICV	Impaired Colour Vision
IOP	Intraocular Pressure
IPVD	Incomplete Posterior Vitreous Detachment

IPL	Inner Plexiform Layer
INL	Inner Nuclear Layer
ILM	Internal Limiting Membrane
IS/OS	Inner Segment/ Outer Segment
LOCS	Lens Opacity Classification System
LD ratio	Light-Dark Ratio
MRT	Mean Retinal Thickness
MP	Microperimetry
MRS	Mean Retinal Sensitivity
NC	Nuclear Cataract
NPDR	Non Proliferative Diabetic Retinopathy
NIDDM	Non-Insulin Dependent Diabetes Mellitus
OPL	Outer Plexiform Layer
ONL	Outer Nuclear Layer
OR	Odds Ratio
PVD	Posterior Vitreous Detachment
PSC	Posterior Subcapsular Cataract
PRL	Photoreceptor Layer
RNFL	Retinal Nerve Fiber Layer
RPE	Retinal Pigment Epithelium
SD-OCT	Spectral Domain Optical Coherence Tomography
SN-DREAMS	Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study
STDR	Sight Threatening Diabetic Retinopathy
S-index	Selectivity index
SPSS	Statistical Package for Social Sciences
TES	Total Error Score
VA	Visual Acuity

Chapter 1

Chapter 1: Introduction and Review of Literature

1.1 Structural and Functional Changes of Retina in Diabetic Retinopathy

Diabetic retinopathy (DR) is a vascular disorder affecting the microvasculature of the retina. It is a major cause of blindness in the working age group worldwide and remains one of the most serious complications of diabetes mellitus (DM).(1,2) Identification of early changes in case of DR is important which can help in the diagnosis and management of disease. Neuronal degeneration is the critical component of DR.(3,4) Various studies report that neuronal changes in the retina take place even before development of microvascular changes.(3,5,6) Ophthalmoscopy, fundus photography and fluorescein angiography (FA) are the common tools to diagnose DR and diabetic macular edema (DME). There is an increasing demand for high-resolution imaging of the ocular tissue to improve the early diagnosis and management of DR. Newer imaging techniques like Spectral domain optical coherence tomography (SD-OCT) with their better resolution are available which aid in assessment of morphological changes.

Visual acuity (VA) is the most widely used measure of macular function. However, in the early stages of the disease VA is not usually affected. A functional assessment of vision is required for the early diagnosis of the disease and follow-up of the patients to understand the progression and effect of various treatment modalities. Various functional tests like Humphrey visual fields, electro-retinogram (ERG) and microperimetry have demonstrated early damage in retinal pathologies. Clinic based studies have assessed these changes among subjects with DR and very few studies assessed neuronal changes in terms of both structural and functional abnormalities among subjects with diabetes but no retinopathy.(5,7–13) However, there is little or no literature assessing structural and functional changes among subjects with diabetes with and without retinopathy in a population based study.

1.2 Review of Literature

1.2.1: Burden of Diabetes Mellitus and Diabetic Retinopathy

Diabetes is one of the largest global health emergencies of the 21st century, affecting over 415 million people world-wide; International Diabetes Federation has estimated that this number would rise to 642 million by 2040.(14) India is home to the second largest number of adults living with diabetes worldwide, after China. Approximately one-third of the people with diabetes develop some degree of diabetes related eye damage or retinopathy. Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study was a population based study, in which we report the prevalence of DM and DR to be 28% and 18% respectively in an urban population in south India.(15) The cohort of this study was evaluated at 4-year follow-up to estimate the incidence of DR, which was reported to be 9%. (Unpublished data) This group also reported the prevalence of the same to be 10.4 and 10.3% respectively in rural population of south India. (16) The sedentary lifestyle and unhealthy food preferences could be the possible reasons for this rural and urban difference.

The awareness about DR is low, both among the diabetics and the diabetic care providers. In a population based study to determine knowledge, awareness and practices (KAP) relating to DR among paramedics in a south Indian population, it was found that over 50% of the respondents were not aware of risk factors for retinopathy. Only one-fifth of paramedics and one-tenth of persons from the community were aware that uncontrolled diabetes was a risk factor for retinopathy. Although 80% of respondents from the community felt that yearly eye examinations were essential, only 43.5% had ever visited an ophthalmologist.(17) This lack of awareness and increased risk of DR among diabetes warrants initiation of mass awareness and screening

programs. Also the timely detection and early management of the condition by routine comprehensive eye examination would reduce the burden of DR in India.

1.2.2: Structural changes among diabetes with and without retinopathy

Colour fundus photography (Figure 1.2.2.1) is a useful tool in the management of diabetic eye disease. It is helpful for documentation of retinopathy as well as counseling the patients. It is also useful for monitoring improvement or progression of retinopathy over time. However standard macular photography cannot confirm clinically significant macular edema (CSME). Winfried Goebel et al(18) compared time domain optical coherence tomography (OCT), retinal thickness analyzer and fundus photography for diagnosing macular edema in 124 eyes of patients with DR and they have reported that OCT seems to be more suitable in the clinical screening for macular edema due to its high sensitivity (> 90%).

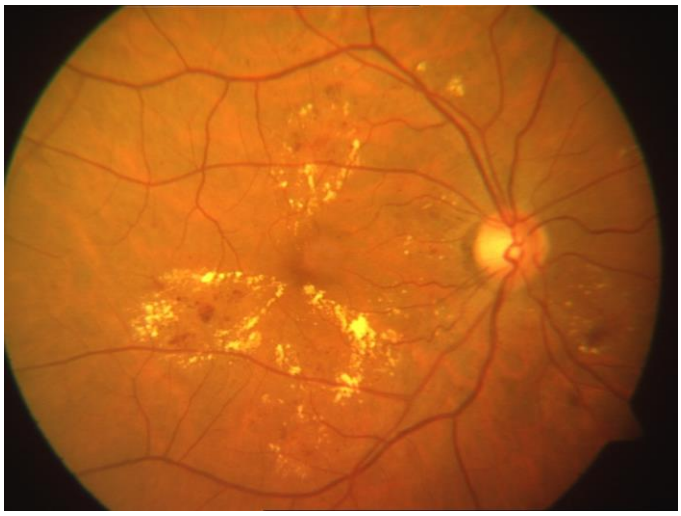


Figure 1.2.2.1: Colour Fundus photography

Optical coherence tomography was first reported in 1991, and since then, this technology has been evolving quite rapidly. Nowadays, OCT is a fundamental diagnostic imaging technique in ophthalmology. It is an essential compliment to ophthalmoscopy and fluorescein angiography in patients with diabetic maculopathy. The conventional time domain OCT provide images with a theoretical axial resolution of approximately 10 µm. SD-OCT is the most recent commercial OCT model and uses a spectrometer as detector of OCT signal or by varying the (narrowband) wavelength of the light source in time producing an axial resolution of 5 µm.(19) Introduction of SD-OCT has improved the image resolution and imaging speed, yielding unprecedented images with improved resolution single frames, volume rendering with 3-dimensional imaging, and mapping of individual retinal layers(20,21) (Figure 1.2.2.2).

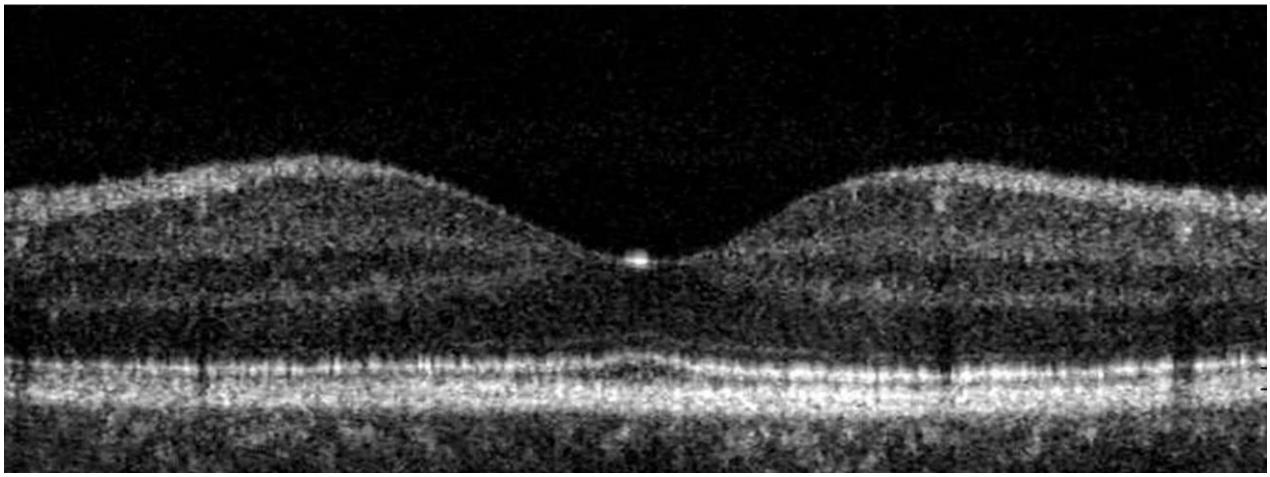


Figure 1.2.2.2: SD-OCT cross-sectional image of normal retina

Previous studies on macular thickness measurements among diabetic subjects with and without retinopathy, obtained using OCT and other instruments such as retinal thickness analyzer have reported variable finding.(5,6,9,22–29) Few studies reported a decreased retinal thickness among diabetic subjects with no retinopathy compared to healthy controls,(5,6,9,22,23) and hypothesized that this was due to the neuronal degeneration in the early stages of DR.(3,4) In contrast, Fritsche et al (24) and Lattanzio et al (25) have reported that retinal thickness was more in diabetic subjects without retinopathy compared to non-diabetic subjects. Others have reported no difference in retinal thickness between subjects with diabetes with minimal or no DR compared to healthy individuals.(19,20) Importantly, previous studies have recruited diabetic subjects from universities or hospital based setup, hence were subject to selection bias. Also the discrepancies might be due to the difference in methodology been followed in various studies. Van Dijk HW et al(23) has stated that retinal thickness measurements based on time domain OCT are not suitable to detect early neuronal degeneration. And they have recommended that increased resolution of SD-OCT and or neuroretinal functional tests can be useful to detect early neural cell defects.

Kim et al(30) reported various morphological patterns of OCT among subjects with DME, which were diffuse retinal thickening, cystoid macular edema, posterior hyaloid traction, serous retinal detachment and tractional retinal detachment. They also reported that retinal thickness varied depending on the type of morphological pattern. Studies also reported reduced VA with increase in the foveal thickness.(31) SD-OCT with its superior delineation enables to evaluate structures like photoreceptor layer, external limiting membrane (ELM) and inner segment and outer segment (IS/OS) junction. Thickness of outer segment of photoreceptors (32) and disruption of IS/OS junction (33) were reported to be associated with VA among subjects with DME.

Vitreoretinal interface and posterior vitreous detachment (PVD) plays an important role in the development and progression of DR. Both clinical (34) and autopsy (35) studies have shown that liquefaction and syneresis of vitreous are more common among people with diabetes. High incidence and early onset of PVD has been reported among subjects with diabetes even without DR.(35,36) Snead et al have reported the prevalence of PVD to be 57% in a randomly selected group of normal subjects.(37) In our previous study we report the prevalence to be 63.3% among subjects with type II diabetes.(38) The high prevalence among subjects with diabetes was reported to be due to the hyperglycemia, which alters the structure and function of the collagen network via increased glycation and crosslinking of the collagen fibrils.(39) Risk factors for PVD included age, gender and axial length.(40,41) There are evidences that progression of no PVD to complete PVD (CPVD) had a protective effect on the development of proliferative diabetic retinopathy (PDR).(42)

1.2.3: Functional changes among diabetes with and without retinopathy

Visual acuity (VA) has been extensively used as an indicator of visual function, and it is also well known that it is affected among subjects with diabetes. However, in the early stages of the disease VA is not usually affected. Moreover, VA measurement provides only limited evaluation of the subject's spatial vision. So evaluation of colour vision, contrast sensitivity (CS) etc. is needed for complete assessment of visual function among diabetic subjects with and without retinopathy.

Several tests are available for the assessment of colour vision; perhaps the best known and widely used panel test is the Farnsworth-Munsell 100 (FM 100) Hue test. Advantage of this test is that the results can be quantitatively scored(43,44) and compared to statistical norms.(45) Studies reported abnormal hue discrimination among subjects with DR.(46,47) Fong et al (46)

have reported that severity of macular edema, age and presence of new vessels were the factors strongly associated with impaired colour discrimination. They also reported that tritan-like defect was prominent and increased in magnitude with increasing severity of macular edema. Hardy et al (48) in their study assessed colour vision abnormalities in uncomplicated type I diabetic patients with angiographically normal retinas have concluded that colour discrimination may be abnormal in these patients before the onset of retinopathy and that colour discrimination losses in diabetes may not be of vascular etiology. Shoji et al (49) in their cross-sectional study among males aged 20-60 years reported that, acquired colour vision impairment was high among type II diabetic patients without DR, but not in subjects with impaired fasting glucose when compared to subjects with normal fasting glucose.

The discriminative ability of the letter contrast sensitivity (CS) using Pelli-Robson chart has been used as an effective screening tool to assess damage to the visual pathway among subjects with diabetes with and without retinopathy.(50) Various hospital based studies reported significantly reduced CS among subjects with diabetes both with and without retinopathy. (50–52) Arend et al(53) have assessed the relationship of foveal microcirculation (perifoveal intercapillary area and size of the foveal avascular zone) to CS function in early DM and concluded that alterations of the perifoveal network are related to selective disturbance of central visual function as measured by CS. Sokol et al (54) assessed CS function at six spatial frequencies (0.5 to 22.8 cycles/ degree) among subjects with insulin dependent (IDDM) and non-insulin dependent (NIDDM) diabetes mellitus with minimal or no visible retinopathy. They reported that subjects with IDDM and no retinopathy had normal CS, subjects with NIDDM and no retinopathy had abnormal CS at only one spatial frequency (22.8 cycles/ degree) and those with NIDDM and background retinopathy had abnormal CS at all spatial frequencies.

Microperimetry (also known as fundus perimetry) has been recently introduced, which allows exact topographic correlation between fundus details and its light sensitivity (differential light sensitivity or retinal threshold). Microperimetry examination would be an ideal tool to measure retinal sensitivity (RS), scotoma size and fixation behavior in subjects with macular pathologies. Studies have reported that macular hard exudates and DME were associated with reduced RS.(10,55–57) These lesions cause the light to be blocked or scattered before it reaches the photoreceptors, suggesting that optical effects are the major cause of sensitivity loss.(56) Yohannan et al(58) in their cross-sectional study reported that the absence of IS/OS junction of photoreceptors was significantly associated with 3.28 dB decrease in retinal point sensitivity in eyes with DME. Our previous study assessing the correlation between structural alterations and RS in morphological patterns of DME revealed that, RS showed better correlation to OCT patterns of DME than the retinal thickness.(59)

Kim et al (60) retrospectively studied the relationship between CS and RS assessed by microperimetry in subjects with DME and reported that CS and RS showed significant correlation in subjects with CSME. They also reported that CS and microperimeter are complementary to each other and are useful tools in evaluation of functional vision.

The study of fixation characteristics, stability and location, has gained increasing interest for visual performance evaluation and rehabilitation in many macular diseases.(61–63) Although various studies agree that macular sensitivity decreases in subjects with DME, data about fixation characteristics are quite contrasting.(57,61,62,64,65) Vujosevic et al (65) in their prospective study evaluated fixation characteristics among patients with DME, reported that fixation location and stability are independent of edema characteristics (diffuse, focal, cystoid,

sponge like, with or without subfoveal neuroretinal detachment), except when subfoveal hard exudates are present.

1.3 Gap in existing research:

Studies on hospital based sample have been done stating the changes that occur in patients with DR using time domain optical coherence tomography. To the best of our knowledge there are no studies assessing the structural changes that occur in diabetic subjects with high resolution spectral domain optical coherence tomography in a population based sample. There is little or no data reporting the functional changes that occur in these patients and also the structural and functional correlation in various stages of DR in a population based study. The other less well investigated cause is neurodegenerative theory. The neurodegenerative changes are apoptosis of several neuronal cells including ganglion, amacrine, horizontal, muller and photoreceptor cells; these changes probably precede microvascular changes. The main outcome of our study is to assess structural changes and then correlate them with the functional changes that occur among subjects with diabetes with and without retinopathy.

1.4 Study Objectives:

1. To elucidate changes in the sensory retina, using spectral domain optical coherence tomography among subjects with diabetes with and without diabetic retinopathy.
2. To assess the functional changes using Pelli-Robson contrast sensitivity chart, FM 100-hue test and microperimeter.
3. To determine the relationship between structural and functional changes of the retina among subjects with diabetes with and without diabetic retinopathy in a population based cohort.

Chapter 2

Chapter 2: Methodology

2.1 Overview of Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study (SN-DREAMS)

Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study (SN-DREAMS I) was a population based cross-sectional study designed to estimate the prevalence of DR in an urban population aged 40 years and above in Chennai, Tamil Nadu. The study design and research methodology of SN-DREAMS I was described in detail elsewhere.(66) The study sampling was based on multistage systematic random sampling. The total population of Chennai was 4.3 million, with 28% of population more than 40 years according to the census of 2001. The computed sample size for this study was 5830. Chennai city was divided into 10 corporation zones of 155 divisions. 10 divisions are randomly selected ensuring that one division per one corporate zone is represented in the sample. To meet the target 600 individuals are enumerated for each division achieving a sample of 6000. SN-DREAMS I was started in 2003 and completed in 2006. Written informed consent was obtained from all subjects and the study was performed in accordance with the tenets of the Declaration of Helsinki. SN-DREAMS and its subsequent follow-up studies were approved by the institutional review board, Vision Research Foundation, Chennai.

SN-DREAMS II was a 4-year follow-up study of SN-DREAMS I performed to assess the incidence of DR and its risk factors. Total 1563 subjects were followed up in this cohort of which 1425 were diabetics and 138 subjects were non-diabetics. These 1563 subjects were invited to the base hospital for comprehensive evaluation including biochemical investigations; 992 (63.46%) responded. Of these 992 (previously diagnosed to have diabetes, 911; non-diabetic from cohort, 81) who visited the base hospital, 12 were excluded as the oral glucose tolerance

test (OGTT) value was less than 200 mg% after 2 hour of oral glucose intake. An additional 22 individuals were excluded because of their ungradable digital fundus photographs. Thus, a total of 958 were analyzed for the incidence of DR. Figure 2.1.1 shows flow-chart for recruitment of SN-DREAMS cohort. Our study is part of SN-DREAMS II.

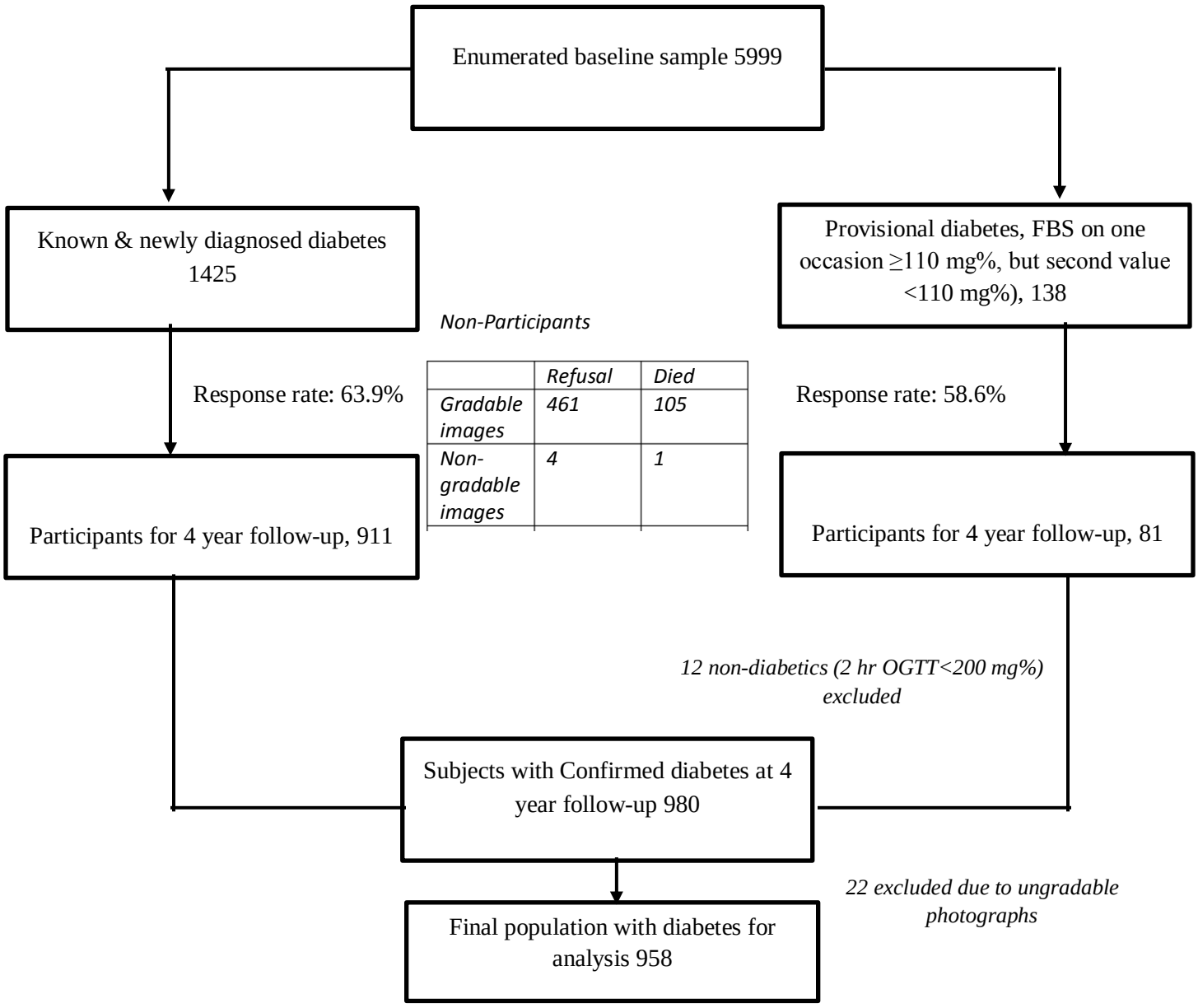


Figure 2.1.1: Flow-chart for recruitment of Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular genetics Study (SN-DREAMS) cohort

2.2 Inclusion criteria

- The subjects included in the study comprised of the subjects with DM with or without clinical evidence of DR.

2.3 Exclusion Criteria

- Subjects associated with vascular or pathological retinal changes other than DR
- Significant media opacities which could impair fundus evaluation
- Subjects with history of any intravitreal injection or any retinal surgery
- Previous history of intraocular laser treatment.
- SD-OCT scans of poor image quality
- Un-reliable test on microperimetry

Based on the above inclusion and exclusion criteria 91 subjects were excluded further, the reasons being, 7 had glaucoma, 1 had corneal opacity, 80 had age related macular degeneration and 3 had optic atrophy. Thus the final sample for the study was 867.

Table 2.1 Distribution of sample based on diabetic retinopathy grading

Fundus grading	Subjects (n)
No DR	692
Mild NPDR	65
Moderate NPDR	56
Severe NPDR	7
PDR	14
CSME	33

Clinical examination was done for all the subjects, including detailed history, biochemical investigations and comprehensive eye examination (Elaborated in Appendix I) which also

included imaging tests: SD-OCT, B-scan ultrasonography and functional tests: Contrast sensitivity using Pelli-robson chart, FM-100 hue colour vision test, Microperimetry.

2.4 Functional Tests

2.4.1 Contrast Sensitivity:

Contrast sensitivity was assessed using a Pelli-Robson chart (Figure 2.4.1.1a) at 1 m distance.(67) The chart consists of 8 lines of letters with different contrast. Each line has 6 letters (2 triplets), first triplet on the left have more contrast than the right triplet. The contrast also decreases downward from line to line. The letters of top left triplet have the highest contrast of 100% and on the bottom right corner have the lowest contrast of 0.6%. Figure 2.6.1.1b shows the logarithmic contrast sensitivity (1/contrast) values. The logarithmic contrast sensitivity value of the last triplet of which at least 2 letters are correctly seen is marked as the result.(68)

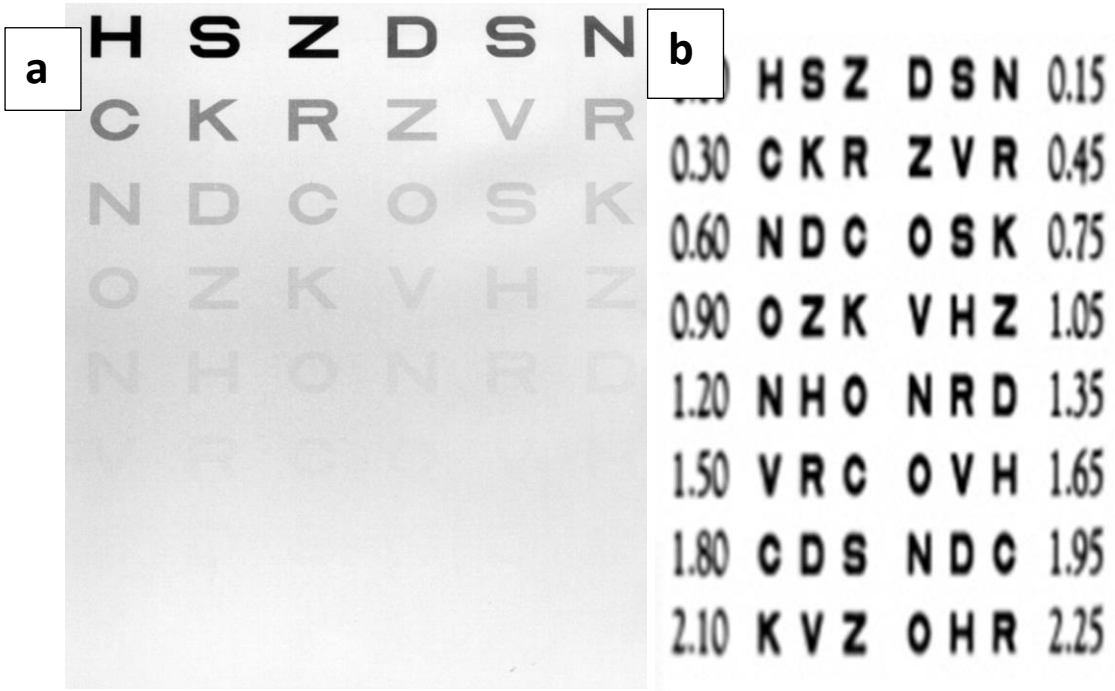


Figure 2.4.1.1: The Pelli-Robson contrast sensitivity test chart

2.4.2 Colour Vision test:

Colour discrimination was assessed monocularly with FM 100-hue test, under the FM 100-hue viewing booth lighting condition (developed by Zahiruddin et al(69)) at a distance of 30 cm with near correction in place. (Figure 2.4.2.1) The test consists of 85 colored caps in a hinged wooden case representing a full range of colors spanning the visible spectrum. The caps are arranged in 4 separate trays, each containing a fixed reference cap at each end. There are 22 caps in one tray and 21 caps in each of remaining 3 trays. The objective of the test was to arrange the caps in a successive order by placing the cap closest in hue to the reference cap and continuing by placing the remaining caps in same fashion. All caps are numbered on the underside for scoring. To make the subjects understand FM 100-hue test and also to remove the effect of learning curve, we prepared a demonstration video which made the task understandable for the subjects and also allowed the subjects to perform test prior to the actual test of which the results are considered for the study.

Figure 2.4.2.1: FM 100-hue test panels

The FM 100-hue test results were analyzed using web-based scoring software designed by Torok B.(70) Total error score (TES) was assessed based on the classical method. The parameters based on moment of inertia method (44) were as follows:

Major and minor radius were derived from the colour difference vectors plotted based on the cap arrangement by the individual subject

Angle identifies the primary axis of colour confusion.

Selectivity index (S-index) quantifies the amount of polarity or lack of randomness in a cap arrangement, calculated from the ratio of major and minor radii.

Confusion index (C-index) quantifies the degree of colour loss relative to a perfect arrangement of caps, and was derived by dividing the length of subject's maximum radius by the maximum radius obtained for a perfect arrangement of caps.

Total error score was calculated from the major and minor radii by obtaining the square root of their sum of squares.

2.4.3 Microperimetry:

The Microperimeter (MP1, Nidek Technologies, Padova, Italy) combines fundus-tracking microperimetry with colour fundus photography in a single instrument. (Figure 2.4.3.1) MP1 works on principle scanning laser ophthalmoscope (SLO). The end product of perimetry and microperimetry exams is a sensitivity map of the examined retina. This is obtained by measuring patient's ability or inability to perceive light of varying intensities projected on different areas of the retina. In conventional perimetry, the stimulated fundus areas are identified by their geometric position with respect to the patient fixation area. The sensitivity map is generated by

observing a live picture of the examined retina and allows, therefore, referring stimuli location to precise anatomical references. Microperimetry is then correlated with the fundus of the patient, reason for which it is also called fundus related microperimetry.

Figure 2.4.3.1: Microperimeter (MP1)

Microperimetry was performed in the mydriatic state using Goldmann size III stimuli, 4–2 double-staircase threshold strategy and a white background with an intensity of 4 apostilbs. An automated program was used, 33 stimulus points were projected in the central 20° of fundus. The retinal image was captured initially, using the infrared camera. Then, the location of the blind spot was confirmed. The blind spot was used to check the false positive responses. The stimuli were projected one at a time (duration of stimuli being 200 ms), and the subject was asked to respond to every stimulus seen by him by pressing a hand held button while the subject concentrates at the centre target (1° cross). A well-defined reference mark in the retina was

chosen by the examiner to help track the saccadic eye movements. The false negative responses were tested once in every minute during the test, to test the reliability of the test results. The data was registered on a colour picture, and the retinal sensitivity was expressed in decibels (dB). Mean retinal sensitivity (MRS) in the central 2°, 8°, 10°, 12°, and 20° was measured. Quadrant wise MRS was measured superonasal, superotemporal, inferonasal, inferotemporal to the fovea by selecting 5 stimuli in each quadrant.

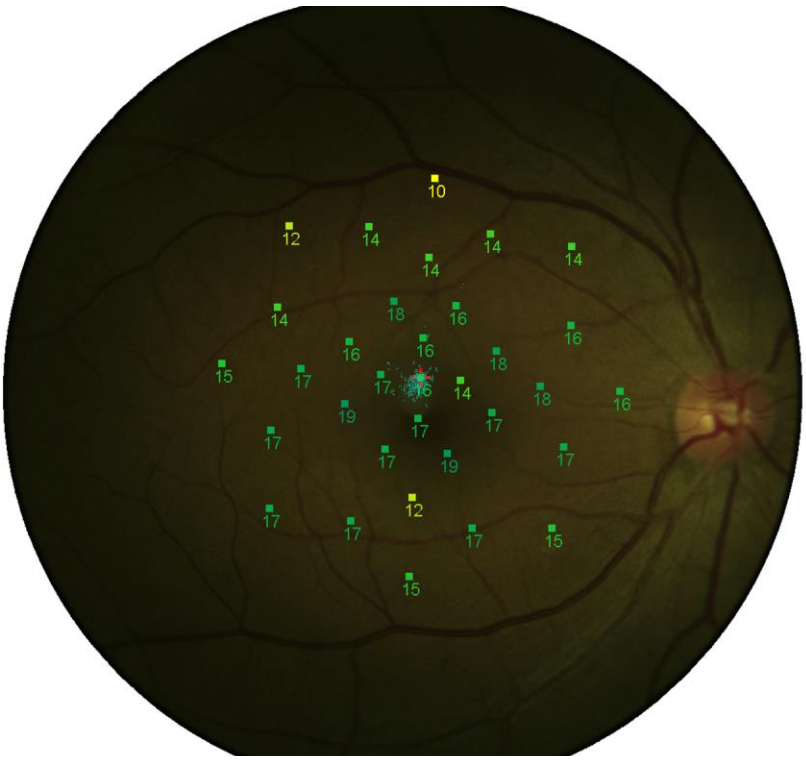


Figure 2.4.3.2: Retinal sensitivity map

Fixation characteristics were measured according to Fujii et al,(71) the standard of central fixation is defined to approximate a 2° diameter (equals, 700 μm) circle centered on the fovea. Eyes with >50% of the preferred fixation points located within central fixation are classified as "predominantly central fixation". Eyes with >25% but <50% of the preferred fixation points

located within central fixation are classified as “poor central fixation”. Eyes with <25% of the preferred fixation points located within the central fixation are classified as “predominantly eccentric fixation”. For evaluating stability of fixation: Eyes with >75% fixation points located within the central 2° are classified as “stable fixation”. If <75% fixation points located within the 2° but >75% fixation points located within the 4° are classified as “relatively unstable fixation”. If <75% fixation points located within the 4° are classified as “unstable fixation”. Scotoma was considered to be present if any of the stimulus point was recorded with 0 or <0 dB.

2.5 Structural Imaging

2.5.1 Spectral Domain Optical Coherence Tomography (SD-OCT):

Spectral Domain Optical Coherence Tomography (SD-OCT, Copernicus, Optopol Technologies, Zawierci, Poland) works on the principle of low coherence interferometry. SD-OCT scans were performed through a dilated pupil while monitoring the reconstructed video image of the central retina. The programs used for the present study were Asterisk scan and 3D scan protocols. For the purpose of this study we used a scan length of 7mm with 6 B-scans and 3,000 A-scans per B-scan through the centre of the fovea for the asterisk scan protocol. The scan acquisition time was 0.8 seconds. 3D scan protocol was used with 7mm scan length with 50 B-scans and 1,000 A-scans per B-scan with the time acquisition of 2.4 seconds. All the measurements were calculated in microns.

The following parameters were noted on SD-OCT:

Central foveal thickness (CFT): This was manually measured as the distance between the internal limiting membrane (ILM) and the anterior surface of the retinal pigment epithelium (RPE).

Photoreceptor layer (PRL) thickness: It was defined as the distance between the ELM which appeared as a thin hyper reflective line on the SD-OCT and the anterior surface of the RPE.

RPE thickness: It was defined as the distance between the inner and outer edge of the RPE layer.

Retinal Nerve Fiber layer (RNFL) thickness: It was measured at 2.5mm nasal to the central fovea by the automated software which provided the distance between the ILM and the outer edge of the RNFL.

Central Subfield thickness (CSFT): This was assessed as the retinal thickness in central 1 mm area of ETDRS quadrants.

Mean retinal thickness was given as the average retinal thickness of all 9 ETDRS quadrants i.e., CSFT, superior, inferior, nasal and temporal quadrants, in the middle 3mm and the outer 6mm ring.

CFT, PRL and RPE thickness were measured manually using the calipers available in the SD-OCT software. RNFL thickness was assessed using an automated measurement technique in the macular area (2.5 mm nasal to fovea).

2.5.2 B-scan Ultrasonography:

Status of posterior vitreous was assessed using B-scan ultrasonography (Ultrascan, Alcon Laboratories, Sinking Spring, Penn. USA). It uses high-frequency sound waves, which are transmitted from a probe into the eye. As the sound waves strike intraocular structures, they are reflected back to the probe and converted into an electric signal. The signal is subsequently

reconstructed as an image on a monitor. The status of the PVD was reported on the basis of the following definitions:

• **No PVD:** When there were no echoes in the vitreous cavity. (Figure 2.5.2.1)

• **Incomplete PVD (IPVD):** When there were characteristic movements of the posterior vitreous, with ocular movements, and an echolucent interval between the retina and the posterior hyaloid with attachment to the disc, macula, or extra macular area. (Figure 2.5.2.2)

• **Complete PVD (CPVD):** When there was characteristic movement of the posterior vitreous, with ocular movements, and an echolucent interval between the retina and the posterior hyaloid with no attachment to the retinal surface. (Figure 2.5.2.3)

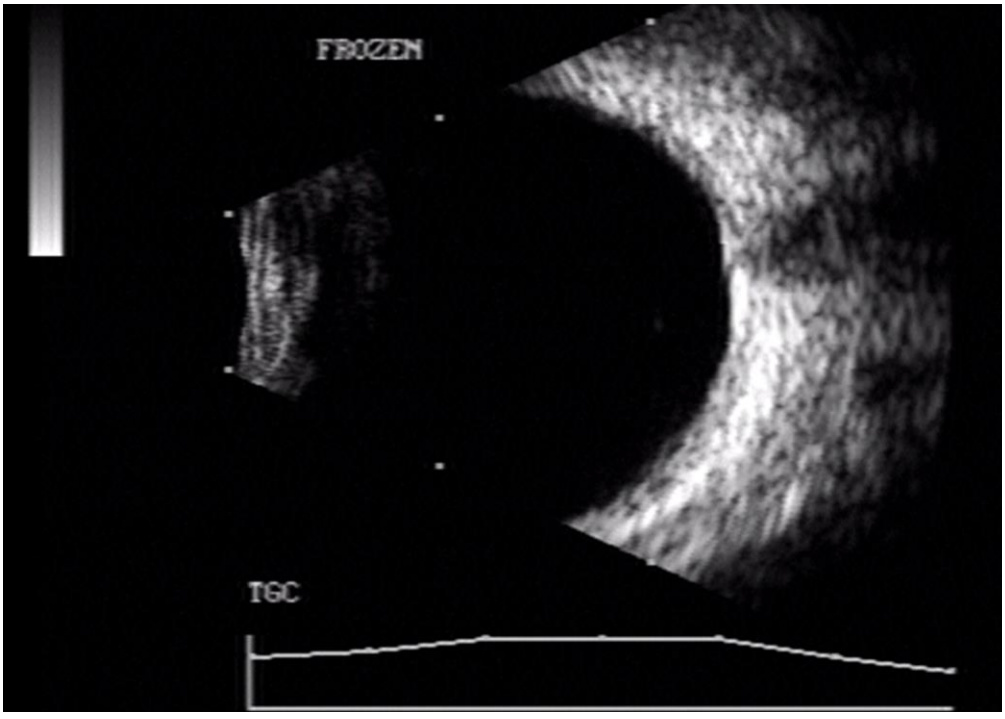


Figure 2.5.2.1: Ultrasound image showing no posterior vitreous detachment

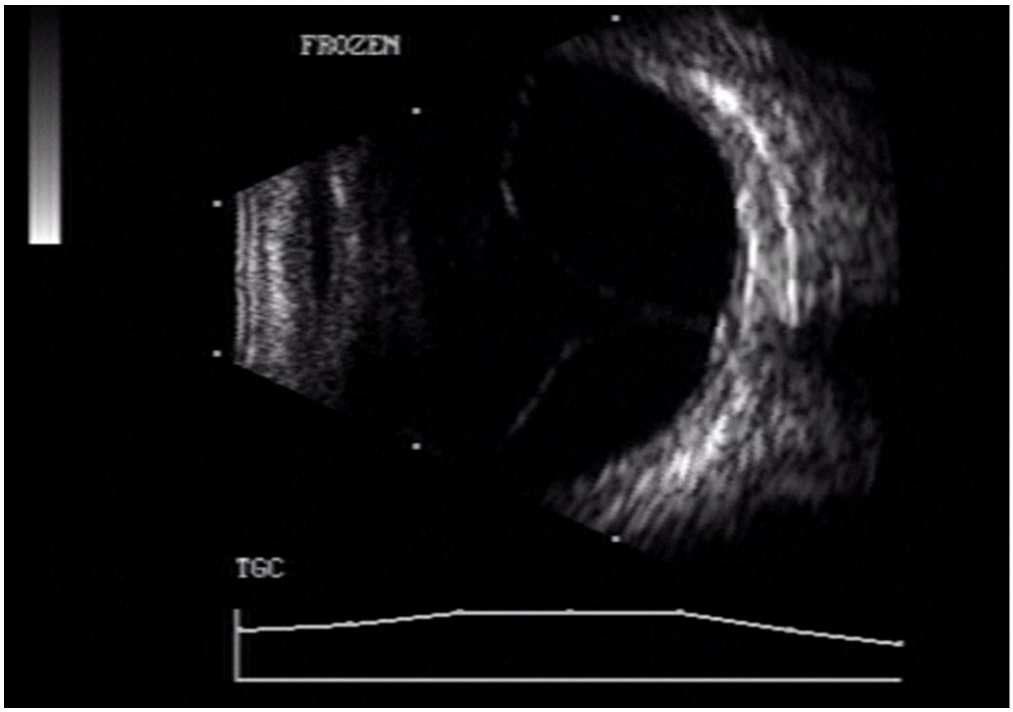


Figure 2.5.2.2: Ultrasound image showing incomplete posterior vitreous detachment

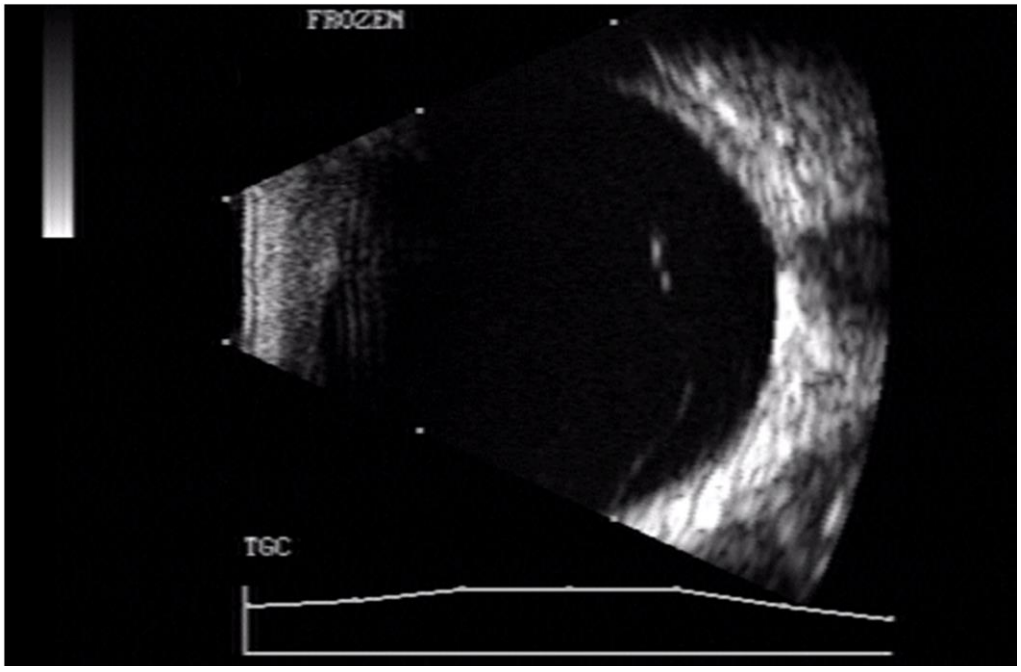


Figure 2.5.2.3: Ultrasound image showing complete posterior vitreous detachment

The following table 2.2 gives the distribution of study sample who have undergone various structural and functional tests

Table 2.2: Distribution of sample based on various tests done

Techniques	Subjects (n)
Optical coherence tomography	668
Microperimetry	357
Colour vision	343
Contrast sensitivity	653
B-scan ultrasonography	615

Chapter 3 and 4 included subjects from a clinic based sample. The details of the clinic based study sample were given in the respective chapters. And chapters 5 to 12 included subjects from population based sample.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences, version 15.0, Chicago, Illinois). Statistical significance was set at the $p < 0.05$ level. In newly diagnosed subjects with diabetes, the duration was considered as 0.

Flow of analysis:

1. Tests for normality were performed.
2. Descriptive analysis was performed
3. Mean comparison of parameters between groups was done using t-test and one-way analysis of variance
4. Comparison of proportional difference was done using chi square test
5. Pearson correlation coefficients were used to find out the association between the parameters.

6. Intra-class correlation was done to assess the intra-observer variability. Bland-and Altman plot was plotted using MedCalc software to see the inter-observer variation

7. Risk factors for various parameters after adjusting for confounding factors was done using multivariate logistic regression.

Chapter 3

Chapter 3: Quantitative SD-OCT Thickness Parameters in Type II Diabetes

3.1 Introduction

Visual impairment in DR can be due to macular edema,(72) vitreous haemorrhage, tractional retinal detachment involving fovea and macular ischemia.(73) The ETDRS study has clearly demonstrated that it is important to differentiate the severity of DR, as moderate non-proliferative diabetic retinopathy (NPDR) carries an 8.6% risk of developing high-risk PDR over a period of one year and severe NPDR carries a 45% risk of developing high-risk PDR.(74) The varying severity is based on morphological features in each stage of retinopathy. The morphological features contribute to the tissue space and thus retinal thickness. These quantitative alterations in the thickness of retinal layers, overall and quadrant wise, can be studied by SD-OCT. These structural changes in various stages of DR have been poorly studied.(24,26,75)

The aim of the present study was to elucidate the changes in the retinal thickness and individual layer thickness among subjects with diabetes with and without retinopathy.

3.2 Methods

This study included subjects with DM, with or without clinical evidence of DR, and non-diabetic subjects from a clinical based sample. Intraobserver variability of retinal thickness parameters was seen by the same observer in a subset of the study sample one week after the initial measurements. For the inter-observer variation in manual measurement of SD-OCT, the thickness parameters were taken by a different observer in a subset of the study sample.

3.3 Results

A total of 251 eyes from 170 subjects were included in this study. The mean age of the study population was 55.34 ± 9.02 years and 56.6% of these were males. The mean duration of DM from diagnosis was 110.9 ± 78.1 months (Range: 1- 444 months). Table 3.3.1 shows the baseline parameters of the study population. We found a significant positive correlation between duration of diabetes and severity of DR ($r = 0.21$, $p = 0.014$).

Table 3.3.1: Baseline characteristics of study population

Group	Sample (no of eyes)	Age (mean ± SD)	Duration of diabetes mellitus (mean ± SD)	Spherical equivalent refractive error (mean ± SD)
Non-diabetic	50	51.7 ± 10.2	NA	0.37 ± 1.0
Diabetes without DR	52	55.9 ± 7.3	79.20 ± 46.1	0.28 ± 1.3
Mild NPDR	50	56.8 ± 9.3	103.88 ± 74.2	0.34 ± 1.4
Moderate NPDR	51	55.6 ± 9.1	128.65 ± 69.2	0.25 ± 1.2
Severe NPDR	28	56.3 ± 7.3	104.00 ± 81.1	0.35 ± 1.3
PDR	20	56.8 ± 9.3	182.11 ± 122	0.18 ± 2.0

DR: Diabetic retinopathy; **NPDR:** Non-proliferative diabetic retinopathy; **PDR:** Proliferative diabetic retinopathy

Table 3.3.2 shows gender difference in thickness parameters. Men had significantly greater CFT, CSFT, RNFL thickness and retinal thickness in all the quadrants of 3mm and 6mm zones compared to women ($p< 0.001$). But no significant difference was found in PRL ($p = 0.91$) and RPE ($p = 0.11$) thicknesses.

Table 3.3.2: Retinal thickness measurements according to gender

	Men Mean ± SD	Women Mean ± SD	*p*
CFT	178 ± 19.63	168 ± 20.96	< **0.001**
PRL thickness	62.30 ± 6.56	62.21 ± 6.07	0.91
RPE thickness	42.28 ± 6.33	40.91 ± 7.40	0.11
RNFL thickness	22.55 ± 6.74	20.14 ± 7.57	**0.008**
CSFT (1mm)	208 ± 23.55	192.47 ± 31.06	< **0.001**
Inner zone (3mm)			
Superior	290.19 ± 23.54	275.83 ± 26.44	< **0.001**
Nasal	279.53 ± 23.03	263.47 ± 24.39	< **0.001**
Inferior	286.55 ± 23.12	267.55 ± 25.80	< **0.001**
Temporal	280.26 ± 22.66	264.79 ± 24.53	< **0.001**
Outer zone (6mm)			
Superior	299.73 ± 26.02	287.37 ± 21.17	< **0.001**
Nasal	310.30 ± 21.17	298.98 ± 18.11	< **0.001**
Inferior	302.01 ± 22.29	289.71 ± 18.53	< **0.001**
Temporal	288.78 ± 25.52	273.67 ± 17.85	< **0.001**

CSFT: Central subfield thickness; **PRL:** Photoreceptor layer; **RPE:** Retinal pigment epithelium; **RNFL:** Retinal nerve fiber layer; **FT:** Foveal thickness

Figure 3.3.1 depicts the quadrant wise retinal thickness in the study population. When the retinal thickness in different quadrants were compared there was no difference found between non-diabetic subjects and subjects with diabetes but no DR. In DR group, all quadrants, had a significantly increased retinal thickness compared to non-diabetic subjects (p= 0.004, p= 0.016, p=0.024, p=0.005 in superior, inferior, nasal and temporal quadrants respectively). Only superior

(p= 0.044) and temporal (p= 0.011) quadrants showed an increased retinal thickness in any DR group when compared with subjects with diabetes but no DR.

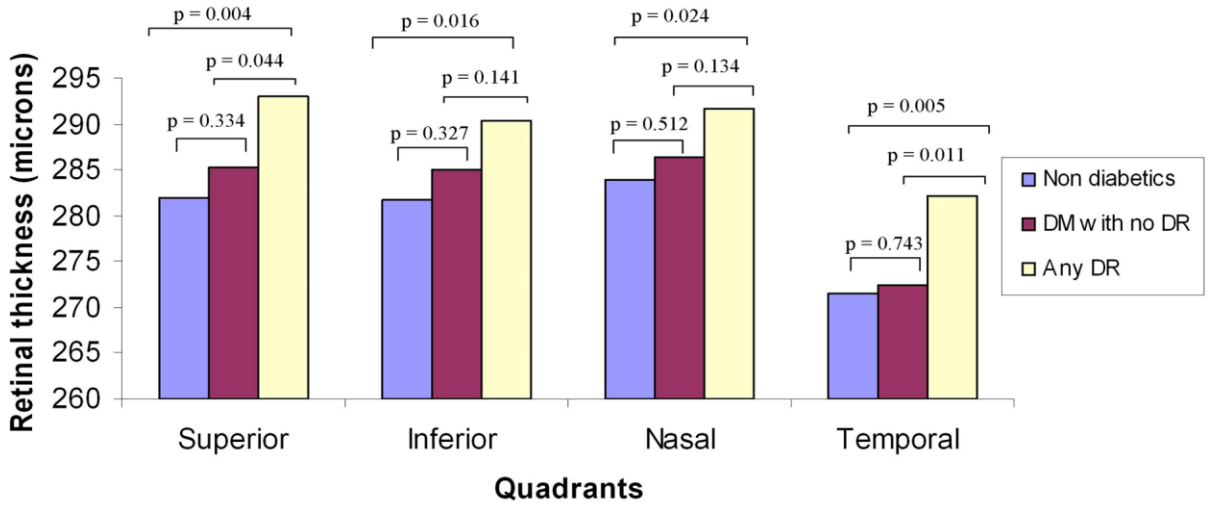


Figure 3.3.1: Quadrant wise retinal thickness

Table 3.3.3 shows the thickness parameters in various DR groups. On comparing the thickness parameters between non-diabetic and diabetic subjects with no DR, the PRL thickness was significantly reduced in diabetic subjects with no DR (non-diabetic - 65.04 ± 4.43 µm, no DR - 62.75 ± 4.82 µm; p= 0.014). On comparison of diabetic subjects with no DR and those with mild and moderate NPDR, CFT was significantly high and the RPE thickness was significantly low in mild and moderate NPDR (CFT: No DR - 167.28 ± 14.56 µm, Mild and moderate NPDR - 177.12 ± 20.99 µm; p= 0.003. RPE thickness: No DR - 43.25 ± 6.90 µm, Mild and moderate NPDR - 40.51 ± 6.14 µm; p= 0.014). PRL thickness was significantly reduced in severe NPDR when compared with mild and moderate NPDR (Mild and moderate NPDR - 61.98 ± 6.64 µm, severe NPDR - 57.53 ± 7.53 µm; p= 0.003). We did not find any significant difference in the CSFT and RNFL thickness among the subgroups.

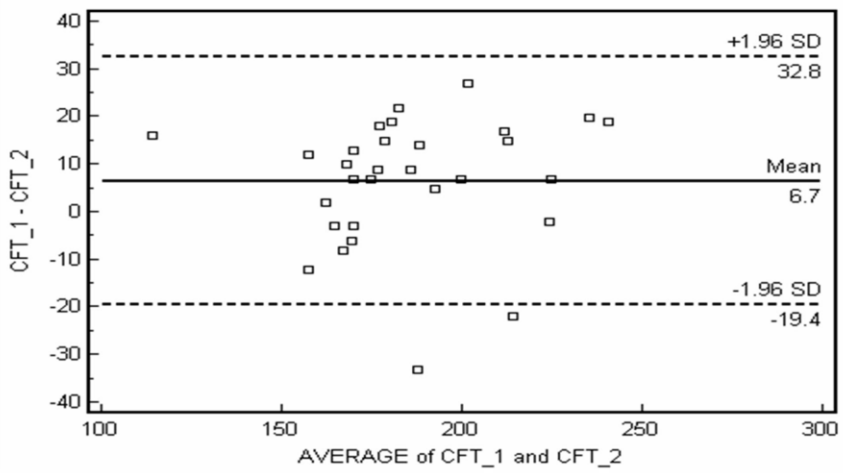
Table 3.3.3: Comparison of retinal thickness parameters in various stages of retinopathy

OCT Parameters	Comparison of non-diabetic and diabetics with no DR			Comparison of diabetics with no DR and mild + Moderate NPDR			Comparison of mild + Moderate NPDR and severe NPDR			Comparison of mild + Moderate + severe NPDR and PDR		
	Normal	No DR	p value	No DR	Mild+ moderate	p value	Mild+ moderate	Severe	p value	Mild+ moderate+ Severe	PDR	p value
CFT	169.64 ± 12.24	167.28 ± 14.56	0.38	167.28 ± 14.56	177.12 ± 20.99	**0.003**	177.12 ± 20.99	175.17 ± 31.08	0.698	176.70 ± 23.43	184.30 ± 25.94	0.186
CSFT	195.02 ± 15.18	194.61 ± 20.15	0.909	194.61 ± 20.15	202.63 ± 31.46	0.097	202.63 ± 31.46	208.21 ± 36.38	0.424	203.84 ± 32.53	220.28 ± 30.81	0.045
PRL	65.04 ± 4.43	62.75 ± 4.82	**0.014**	62.75 ± 4.82	61.98 ± 6.64	0.46	61.98 ± 6.64	57.53 ± 7.53	**0.003**	61.02 ± 7.06	62.10 ± 7.02	0.523
RPE	45.58 ± 5.69	43.25 ± 6.90	0.296	43.25 ± 6.90	40.51 ± 6.14	**0.014**	40.51 ± 6.14	37.82 ± 7.33	0.052	39.93 ± 6.49	39.05 ± 6.67	0.594
RNFL	22.60 ± 5.53	21.19 ± 7.77	0.909	21.19 ± 7.77	21.65 ± 7.01	0.711	21.65 ± 7.01	21.21 ± 8.85	0.783	21.56 ± 7.42	19.30 ± 7.85	0.211

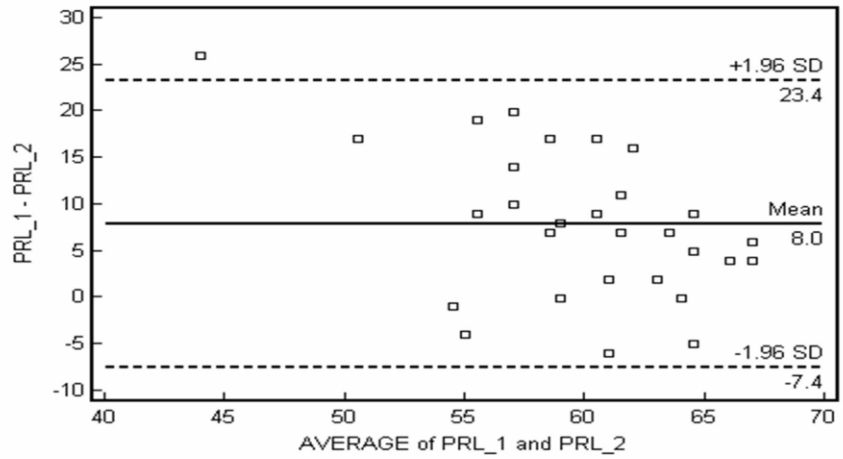
DR: Diabetic Retinopathy; NPDR: Nonproliferative Diabetic Retinopathy; PDR: Proliferative Diabetic Retinopathy; CFT: Central Foveal Thickness; CSFT: Central Subfield Thickness; PRL: Photoreceptor Layer; RPE: Retinal Pigment Epithelium; RNFL: Retinal Nerve Fibre Layer.

Intra-observer repeatability was found to be good in measuring the SD-OCT outcomes with intraclass correlation of 0.99 for CFT, 0.635 for PRL thickness and 0.805 for RPE thickness. Figure 3.3.2 shows the Bland-Altman plot for various thickness parameters. The mean difference in the CFT measurements was 6.7 µm with limits of agreement ranging from -19.4 to 32.8 µm. The mean difference in the PRL thickness was 8 µm with 95% limits of agreement between -7.4 to 23.4 µm; the mean difference in RPE thickness was -2.2 µm with limits of agreement ranging from -9.1 to 4.7 µm.

Bland-Altman plot of CFT measured by 2 observers



Bland-Altman plot of PRL measured by 2 observers



Bland-Altman plot of RPE measured by 2 observers

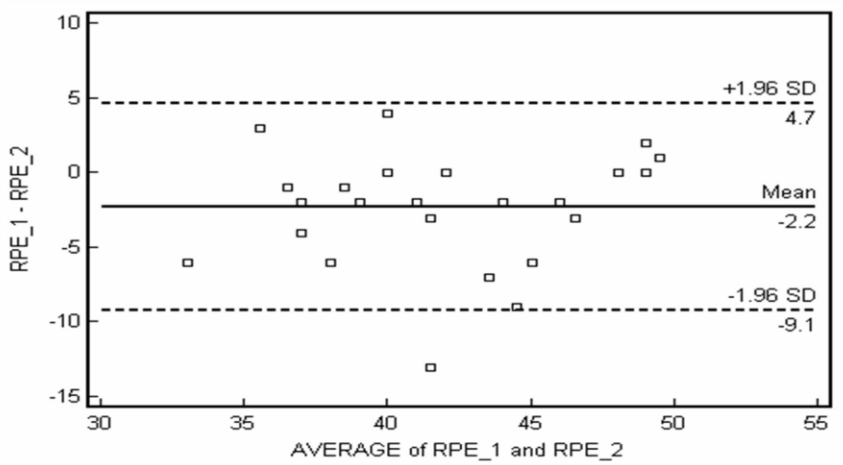


Figure 3.3.2: Bland-Altman plot for SD-OCT parameters

3.4 Discussion

The morphological changes in various stages of DR are reflected by the differences in the retinal thickness in total or thickness of individual layers. Post hoc power analysis revealed 92.3% power in estimating the difference of foveal thickness between subjects with and without retinopathy. Yang and Forooghian et al have reported the changes in DME.(76,77) Significant difference in retinal thickness was reported in various stages of DR using stratus OCT.(23,78) Our group also studied the neuronal changes that occur in diabetic subjects who did not have clinical evidence of DR.(5)

Increased retinal thickness in men was reported in healthy individuals in the study done by Wong et al.(79) Bressler et al(26) in their study reported that in type II diabetes subjects, CSFT was significantly greater in the retina of men with mean of 209 ± 18 µm and it was 194 ± 23 µm in women which was similar to our study results. But they did not find any difference in the inner subfield and outer subfield retinal thickness which was similar to our study results.

Significant difference in the retinal thickness between diabetic subjects with and without DR was only found in the superior and temporal quadrants. These findings make the superior and temporal quadrants the most likely regions where the earliest and clinically non detectable changes in retinal thickness caused by DR can be detected. Our results were similar to the study done by Schaudig UH,(80) in which they found the superonasal quadrants to have the earliest DR changes in terms of changes in the retinal thickness values. This could happen in an area where the retinal thickness and density of ganglion cell bodies are higher, vascular damage resulting in edema will produce thickening, mainly in a posterio-anterior direction whereas in an area with lower density of cells it will take a higher amount of edema to produce the same amount of thickening. We identified that in all cases the nasal quadrant was thicker than the

temporal. This was consistent with the anatomical relationship of the converging of nerve fibres with the optic disc.(81)

An increase in retinal thickness was found in subjects with mild and moderate NPDR when compared to diabetic subjects with no DR. PRL thickness was significantly reduced in diabetic subjects with no DR compared to non-diabetic subjects and also in severe NPDR group compared to mild and moderate NPDR. RPE thickness was significantly reduced in diabetic subjects with mild and moderate NPDR compared to diabetic subjects with no retinopathy. Recent evidence suggests that the selective thinning of inner retinal layers supports the concept of early neurodegenerative component in patients with minimal or no DR.(5,23,82,83) Sufficient evidence also comes from the animal studies that thickness of the inner retinal layers decreases in diabetic condition, indicating early neuronal degeneration.(84,85)

Earlier studies have assessed RNFL thickness in the peripapillary area using OCT, scanning laser polarimetry etc., and reported that the RNFL thickness was significantly reduced with severity of DR,(22,86) however we measured RNFL thickness in the macular area and did not find any difference in RNFL thickness between the groups of DR. This suggests that RNFL thickness did not vary among the subjects with diabetes and with DR in the macular area.

In summary, this study demonstrates a loss of photoreceptor layer thickness in diabetic subjects which support the concept of neuronal degeneration. We also report that superior and temporal quadrants are the most likely regions where the earliest and clinically non detectable changes in the retinal thickness can be detected. These results may help in close monitoring of diabetic subjects before developing DR.

Chapter 4

Chapter 4: Qualitative Spectral Domain Optical Coherence Tomography Characteristics in Diabetic Retinopathy

4.1 Introduction

Identification of early changes in cases of DR is important which can help in the diagnosis and management of disease. Newer imaging techniques like SD-OCT with their better resolution are available which aid in assessment of morphological changes.(87) Ophthalmoscopy, fundus photography and fluorescein angiography (FA) are the common tools to diagnose DR and DME. There is an increasing demand for high-resolution imaging of the ocular tissue to improve the early diagnosis and management of DR. This study aims to report the appearance of various lesions in DR using SD-OCT.

4.2 Methods

Two hundred and eighty seven eyes of 199 subjects were included in this study. The study sample included subjects with DM with or without clinical evidence of DR and non-diabetic subjects from a clinical based sample. The structural changes in cases of subjects with different stages of DR were analyzed on grey scale images based on hyper and hypo reflectivity and also the location of the lesions. We classified the stages of posterior vitreous detachment (PVD) based on the SD-OCT findings. Absence of PVD was diagnosed when the posterior hyaloid was not detected on SD-OCT scan of the macula. IPVD was diagnosed when the posterior hyaloid remained partially attached to the macula on at least one SD-OCT scan. CPVD was diagnosed when posterior hyaloid is visible in the vitreous cavity without any attachment to the macula on all the scans on SD-OCT. CPVD was also confirmed with the indirect ophthalmoscopic examination if the posterior hyaloid echo was not visible in the SD-OCT scan.

4.3 Results

The mean age of the study sample was 55.4±8.9 years. The mean duration of DM from diagnosis was 112.1±76.6 months. Table 4.3.1 shows the quantitative parameters of various lesions in different stages of DR. Most frequently found lesions in SD-OCT are hard exudates and hemorrhages followed by cystic spaces.

Table 4.3.1: Distribution of lesions in eyes with various stages of DR

Stages of DR	Microaneurysms	Hard exudates	Hemorrhages	CWS	CNP areas	Cystic spaces	NVE	ERM
Mild	6	7	6	0	0	1	0	2
Moderate	1	44	17	4	0	6	0	5
Severe	0	24	8	9	1	5	0	5
PDR	1	15	7	3	1	3	3	0
DME	0	34	12	2	0	22	0	4
Total	8	124	50	18	2	37	3	16

DR: Diabetic Retinopathy; **CWS:** Cotton wool spots; **CNP:** Capillary non perfusion areas; **NVE:** Neovascularization elsewhere; **ERM:** Epiretinal membrane; **PDR:** Proliferative diabetic retinopathy; **DME:** Diabetic macular edema

Table 4.3.2 shows qualitative parameters of these lesions in terms of SD-OCT appearance and layers involved. Four morphological patterns of hemorrhages seen on SD-OCT were oval shaped hyper reflective echos surrounded by hypo reflectivity, medium to high reflective echos causing shadow in the inner retina, high reflective echos at RNFL and ganglion cell layer causing shadow in case of flame shaped and organized high reflective membrane at vitreoretinal interface causing shadow in case of subhyaloid hemorrhage.

Table 4.3.2: Appearance of diabetic retinopathy lesions on SD-OCT and layers involved

Lesion	Pattern (no of eyes)	Layers Involved
Microaneurysms	Medium to high reflective echos causing shadow (8)	OPL,INL,IPL,GCL,RNFL
Hard exudates	High reflective echos causing shadow (124)	ONL,OPL,INL,IPL
Hemorrhages	**1.**Oval Shaped Hyper reflective echos surrounded by hypo (25)	ONL,OPL,INL,IPL,GCL,RNFL
	2.Medium to high reflective echos causing shadow in the inner retina (16)	
	3. High reflective echos at RNFL and GCL causing shadow in case of flame shaped (8)	
	4. Organized high reflective membrane at Vitreoretinal interface causing shadow (1)	
CWS	Hyperreflective nodular lesions (18)	RNFL,GCL
CNP areas	Thinning of inner retinal layers (2)	----
Cystic spaces	Optically empty spaces (37)	ONL,OPL,INL,IPL,GCL
Neovascularization	Medium reflective pre retinal membrane causing shadow (3)	Vitreoretinal interface
ERM	Hyper reflective band on the inner surface of the retina (16)	----

ILM: *Internal limiting membrane,* ***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,* ***ERM:*** *Epiretinal membrane,* ***CNP****: Capillary non perfusion,* ***CWS:*** *Cotton wool spots*

Prevalence of IPVD was more in subjects with DR (Figure 4.3.1). In subjects with macular edema the prevalence of IPVD was higher i.e., 55.6 % and no PVD was found in 44.4 % and we did not see any cases in macular edema with CPVD.

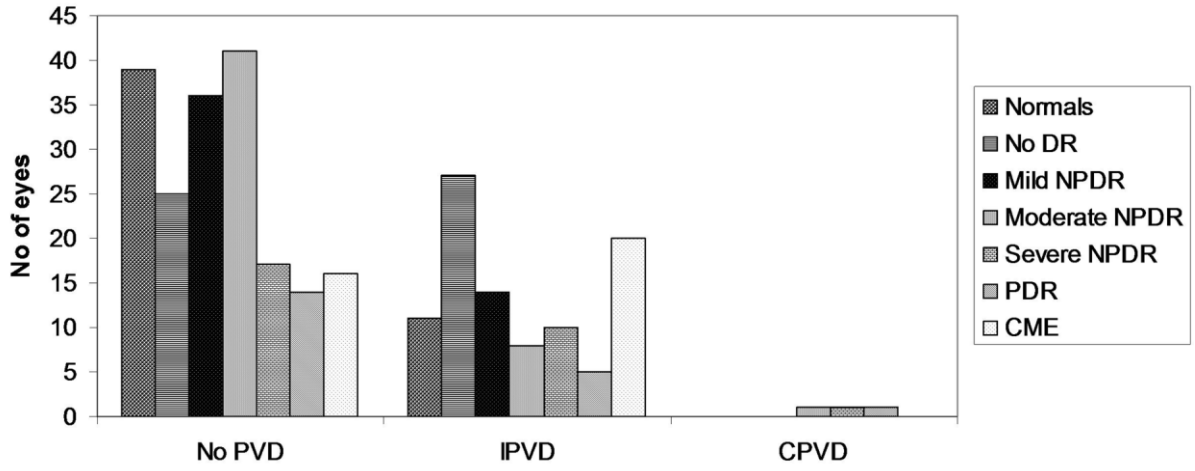


Figure 4.3.1: PVD status in various stages of diabetic retinopathy

4.4 Discussion

In our study we reported appearances of the DR lesions in SD-OCT. Microaneurysms are saccular outpouchings from the sides of cellular capillaries, both on the arteriolar and the venular side. On SD-OCT, they appear as very small, medium to high reflective echos causing minimal shadow in the inner retinal layers (Figure 4.4.1A). Hard exudates are lipoprotein deposits and these appear as very high reflective echos (Figure 4.4.1B) on SD-OCT which cast a shadow on the posterior layers making us difficult to visualize the underlying layers. These hard exudates are present in inner and outer, nuclear and plexiform layers.

Cystic spaces appear as optically empty spaces (Figure 4.4.1C) and these involve the layers from outer nuclear layer (ONL) to the ganglion cell layer (GCL). Histopathologic studies have suggested that the development of macular edema is initiated by fluid accumulation within muller cells. If the accumulation continues, or remains chronic, then at some point death of the

muller cells occurs and may result in the formation of large cystoid cavities, or cystoid macular edema.(30)

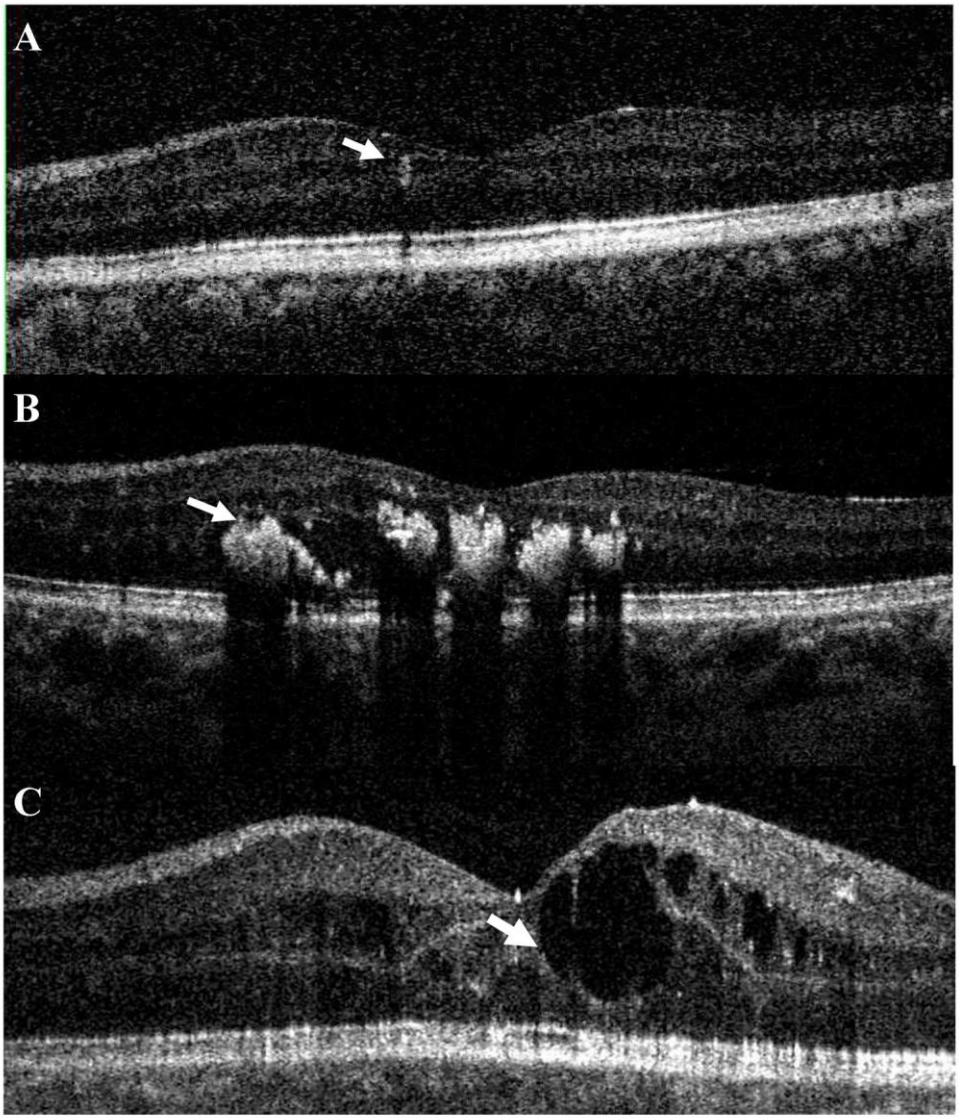


Figure 4.4.1: Lesions on SD-OCT. A: Microaneurysm, B: Hard exudates causing shadowing, C: Cystic spaces

Hemorrhages can be located pre-, intra- or sub-retinal. Various patterns of the hemorrhages can be observed by SD-OCT. In our study we observed 4 different patterns. Most commonly

observed pattern was oval shaped hyper reflective echo surrounded by hypo reflective echo in the inner retinal layers causing shadow and this involves ONL, outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL) and GCL (Figure 4.4.2A). This pattern of appearance may be due to the resolving stage of hemorrhages. Another pattern was medium reflective echos causing shadowing in the inner retina. On ophthalmoscopy when the hemorrhages were present in the RNFL they appear as flame shaped and in SD-OCT these appear as high reflective spots at the RNFL layer causing shadowing. These should not be misinterpreted as the normal blood vessels in the retina which also appear as high reflective echos in the RNFL causing shadowing. In case of sub-hyaloid hemorrhage, SD-OCT shows the presence of a high reflective lesion consistent with blood which is localized and causing shadowing of the underlying retinal layers which is similar to the findings reported in the case reported by Punjabi et al.(88) Due to the collection of blood in the most dependent position of the sub-hyaloid space with the gravity, the horizontal level of the blood is seen as the straight line in SD-OCT, beyond which it causes shadowing on the inner retinal layers. In figure 4.4.2B small arrow shows a thin bright line above the ILM that approximates the organized blood. This could possibly be fibrous tissue that forms a capsule around the blood, but the exact nature of this bright band is unknown.

On SD-OCT cotton wool spots appear as hyperreflective, nodular or elongated lesion in the RNFL (Figure 4.4.2C) and also involving the GCL, which cast shadow on the posterior layers. This appearance is assumed to reflect focal swelling of the nerve fibres,(89) which is due to intracellular fluid and organelles accumulated secondary to interrupted axoplasmic flow.

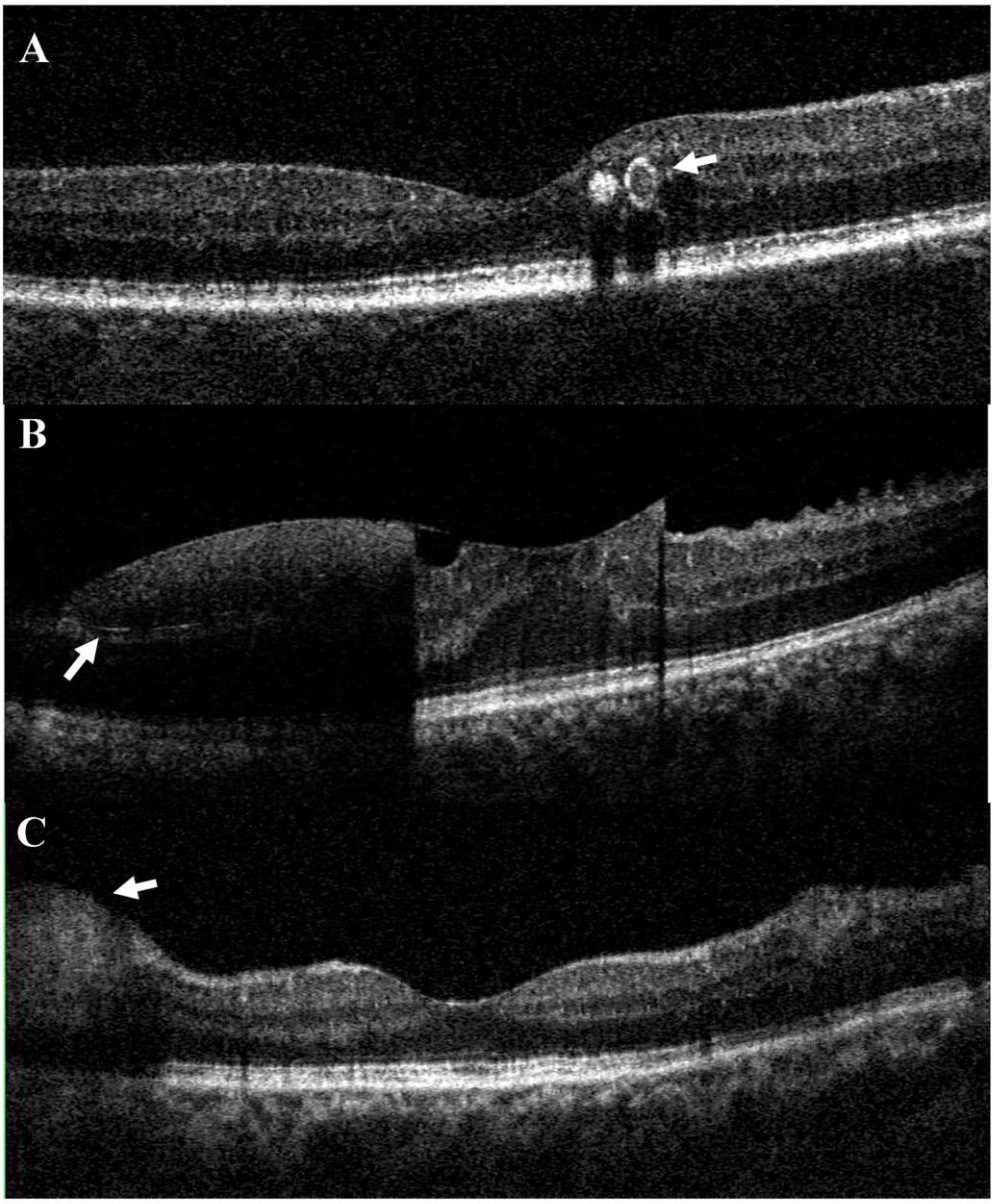


Figure 4.4.2: Lesions on SD-OCT. A: Intraretinal hemorrhage, B: Pre-retinal hemorrhage causing shadow, C: Cotton wool spots

Only in two eyes the SD-OCT was able to pick up the characteristic appearance of capillary non perfusion (CNP) area. SD-OCT showed a thinning of inner retinal layers (Figure 4.4.3B) than the normal retina. This might be due to the reason that the inner retinal layers may be particularly at risk to hypoxic insult because they are supplied with oxygen from the retinal vasculature, which

is relatively sparse compared with the choroidal circulation, which supplies most of the outer retina.(90)

Proliferative DR is characterized by either neovascularization of the disc or elsewhere. Preretinal neovascularization on SD-OCT can be detected as a preretinal high reflective membrane occasionally causing shadow (Figure 4.4.3A). This appearance is due to the presence of some amount of fibroglial tissue.(91)

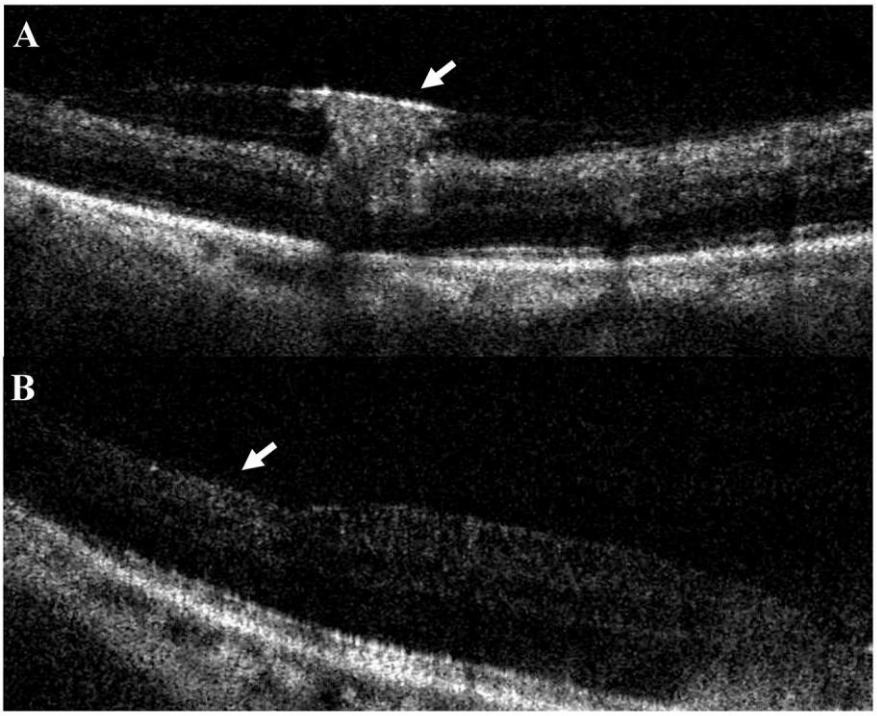


Figure 4.4.3: Lesions on SD-OCT. A: Neovascularization, B: Thinning of retinal layers in capillary non perfusion area

SD-OCT brings new insights into morphological changes of the retina in DR. It enhances the ability to exactly identify the epiretinal membranes (ERM), vitreomacular traction and the

posterior hyaloid status. In our study IPVD i.e., with macular attachment was the most frequent condition found in subjects with macular edema (55.6 %) which was similar to the study results of Gaucher et al.(92) On SD-OCT, the posterior hyaloid was thin and minimally reflective. The high prevalence of IPVD might have been induced by the DME itself. The breakdown of inner blood-retinal barrier in cases of DME might have led to the accumulation of cytokines or other mediators in the posterior vitreous cortex, which might have triggered the PVD. Another reason might be that the IPVD with macular attachment may directly contribute to the development of DME.

In subjects with DR, secondary ERM can develop due to the epimacular proliferating fibroglial tissue. It appears as a hyperreflective band on the inner surface of the retina. In the starting stage it has a global retinal adherence later it causes tractional force on the retina leading to macular edema and due to it discontinuous attachment to the retinal surface causes ILM folds.

To conclude structural abnormalities in DR can be detected and characterized by SD-OCT. It compliments FA in understanding the pathology and managing it accordingly.

Chapter 5

Chapter 5: Foveal slope measurements in diabetic retinopathy: Can it predict development of sight-threatening retinopathy?

5.1 Introduction

Fovea is the specialized region of human retina that drives majority of our visual function.(93) Variation in foveal shape is related to the structural alterations of the retinal layers. Various parameters of foveal structure have been found to be altered in subjects with DR (94–96) and age-related macular degeneration.(97) Although foveal thickness has been used as marker of structural changes in various retinal diseases, there are evidences of using other foveal parameters such as foveal diameter, foveal slope, and foveal depth to assess the structural integrity of macula.(98)

It is known that the foveal avascular zone (FAZ) diameter enlarges in DR and further increases with severity of retinopathy due to the capillary dropout.(95,96) Early neuronal degeneration has also been reported in diabetic subjects even with no retinopathy.(5,6) Dubis et al(99) have shown a strong relationship between FAZ and foveal pit morphology. Foveal slope measurement has been tried in macular diseases such as macular hole(98) and age-related macular degeneration,(97) and correlated with disease prognosis and macular pigment optical density.(100)

To the best of our knowledge, no study has looked into the foveal slope profile in subjects with diabetes and DR. The aim of this study was to assess the foveal slope in subjects with type II diabetes in a population-based cohort and to correlate foveal slope with visual function.

5.2 Methods

From the follow-up study of SN-DREAMS II, of the 867 subjects, 668 subjects who underwent SD-OCT were included in the analysis. Retinal thickness was measured using SD-OCT and retinal thickness was calculated automatically using inbuilt topographic mapping software. Central subfield thickness (CSFT) was noted. The temporal, superior, inferior, and nasal subfield thicknesses were noted in 3 mm ring. Foveal slope in all four quadrants was calculated at a distance of 500 μm from the foveal center (Figure 5.2.1). For example, foveal slope in nasal quadrant was calculated as the difference between nasal quadrant thickness in the 3 mm ring and CSFT divided by 500 μm, which is the distance of nasal quadrant thickness from foveal center.

Slope angle = Difference in thickness/Horizontal distance

Slope (degrees) = $\tan^{-1}$ (Slope angle)

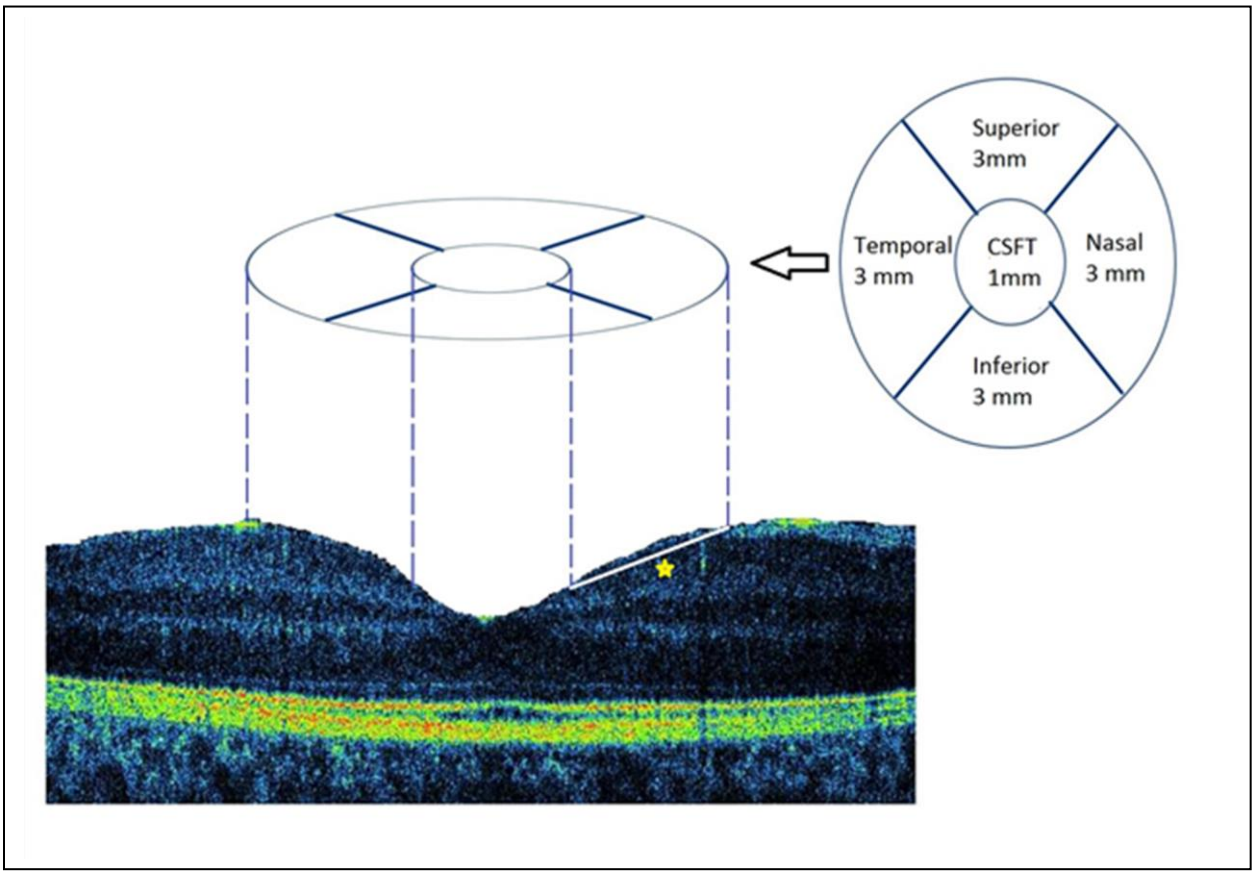


Figure 5.2.1: Measurement of foveal slope in all four quadrants

5.3 Results

The average foveal slope values among subjects with diabetes in superior, inferior, temporal, and nasal quadrants were 8.94 ± 3.04°, 8.71 ± 3.14°, 7.86 ± 2.60°, and 8.07 ± 2.72° respectively. Table 5.3.1 shows the foveal slope characteristics among the study subjects with varying severity of DR. In general, there is a trend of shallow foveal slope in all quadrants with increase in the severity of DR. Shallow foveal slope was found in subjects with STDR in inferior quadrant (STDR: 7.33 ± 6.26 vs controls: 10.31 ± 3.44; p = 0.021) when compared to non-diabetic subjects and in superior (STDR: 7.62 ± 5.81 vs No DR: 9.11 ± 2.82; p = 0.033), inferior (STDR:

7.33 ± 6.26 vs No DR: 8.81 ± 2.81; $p = 0.048$), and temporal quadrants (STDR: 6.69 ± 5.70 vs No DR

: 7.97 ± 2.33; $p = 0.030$) when compared to subjects with no DR.

Table 5.3.1: Foveal slope characteristics among the study sample

	Non-diabetic		No DR		Non-STDR		STDR		Trend *p*
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Duration of diabetes (years)	NA	NA	7.62 ± 5.18	0 to 34	12.59 ± 7.56	4 to 37	14.27 ± 5.64	4 to 24.83	< **0.001**
Superior slope	8.51 ± 2.35	4.01–12.61	9.11 ± 2.82	(–3.44) to 19.48	8.55 ± 2.78	(–5.73) to 13.75	7.62 ± 5.81†	(–14.90) to 14.90	**0.019**
Inferior slope	10.31 ± 3.44	7.45–17.76	8.81 ± 2.81	(–1.72) to 25.21	8.50 ± 3.14	(–6.88) to 15.47	7.33 ± 6.26*†	(–18.91) to 14.32	**0.012**
Temporal slope	8.24 ± 2.07	5.73–13.18	7.97 ± 2.33	(–2.86) to 16.04	7.62 ± 2.18	1.15 to 14.32	6.69 ± 5.70†	(–17.19) to 13.75	**0.027**
Nasal slope	9.70 ± 4.08	4.01–18.91	8.14 ± 2.52	(–2.86) to 21.20	7.69 ± 2.60	(–8.02) to 14.32	7.41 ± 4.65	(–7.45) to 16.62	**0.03**

* Significant when compared with non-diabetics; †significant when compared with no DR

DR: Diabetic retinopathy, **STDR:** Sight-threatening diabetic retinopathy

Table 5.3.2 shows the distribution of average foveal slope among the subject's demographic and systemic factors. Foveal slope was significantly shallow among the older age groups in subjects with no DR ($p < 0.001$) and non-STDR ($p = 0.027$) and was steeper in subjects with poor glycemic control ($p = 0.004$) and in subjects with no DR. No difference in foveal slope was found among the demographic and systemic factors in subjects with non-STDR and STDR.

Table 5.3.2: Average foveal slope and its relation with demographic data

Variables	No DR *n* = 532	*p*	Non STDR *n* = 102	*P*	STDR *n* = 34	*P*
Mean slope	8.50 ± 2.25		8.09 ± 2.23		7.26 ± 4.46	
Age (years)						
40–49	9.09 ± 2.30		8.36 ± 2.38		7.45 ± 0.62	
50–59	8.82 ± 1.93	**< 0.001**	8.16 ± 1.74	**0.027**	7.44 ± 4.50	0.951
60–69	8.01 ± 2.30		8.52 ± 1.69		6.92 ± 5.12	
≥70	7.27 ± 2.59		6.31 ± 3.69		NA	
Gender						
Men	8.63 ± 2.41	0.179	8.11 ± 2.46	0.902	7.53 ± 3.57	0.64
Women	8.37 ± 2.04		8.05 ± 1.91		6.77 ± 5.92	
Duration of diabetes						
≤ 5 (years)	8.57 ± 2.25	0.582	8.53 ± 1.78	0.363	9.02 ± 2.15	0.409
> 5 (years)	8.46 ± 2.25		7.99 ± 2.32		7.03 ± 4.66	
HbA1c						
Normal (<5.6)	7.85 ± 2.16		9.02 ± 1.36		10.89 ± 4.46	
Good to fair (5.6–8.0)	8.48 ± 2.27	**0.004**	7.83 ± 1.98	0.287	6.29 ± 5.35	0.346
Poor (≥8.1)	8.93 ± 2.25		8.25 ± 2.70		7.69 ± 3.47	

Table 5.3.3 shows gender-wise comparison of thickness and slope parameters among the study subjects. Mean retinal CSFT and all the quadrants of inner 3mm ring of ETDRS subfields were significantly thicker in males compared to females. But we did not find any significant difference in foveal slope in all the quadrants between the genders.

Table 5.3.3: Comparison of thickness and slope parameters among men and women

OCT variables	Male	Female	*P*
CSFT	196.98 ± 31.94	187.17 ± 29.53	**< 0.001**
Inner ring superior quadrant	275.87 ± 33.45	264.37 ± 27.56	**< 0.001**
Inner ring inferior quadrant	273.49 ± 30.77	262.05 ± 29.87	**< 0.001**
Inner ring temporal quadrant	266.17 ± 27.74	254.90 ± 26.79	**< 0.001**
Inner ring nasal quadrant	268.53 ± 34.23	255.46 ± 28.57	**< 0.001**
Superior slope	9.02 ± 3.05	8.85 ± 3.05	0.467
Inferior slope	8.77 ± 3.47	8.57 ± 2.66	0.391
Temporal slope	7.92 ± 2.79	7.76 ± 2.35	0.423
Nasal slope	8.20 ± 2.71	7.84 ± 2.62	0.089

Table 5.3.4 shows Pearson and partial correlation between foveal slope and ocular variables. Foveal slope was significantly and inversely correlated with increase in age, increase in duration of diabetes, BCVA, colour vision, refractive error, and CSFT. Significant positive correlation was found between foveal slope and contrast sensitivity. However, when adjusted for all the parameters, partial correlation revealed significant relationship of foveal slope with increase in age ($r = –0.241$; $p < 0.001$) and CSFT ($r = –0.542$; $p < 0.001$).

Table 5.3.4: Correlation between foveal slope and ocular parameters

	Foveal slope Pearson correlation		**Foveal slope Partial correlation**	
	R	***P***	***R***	***P***
Age (years)	-0.231	< **0.001**	-0.241	< **0.001**
Duration of diabetes (years)	-0.112	**0.004**	-0.027	0.678
Visual acuity (log MAR)	-0.122	**0.002**	0.007	0.918
Colour vision (TES)	-0.162	**0.009**	-0.059	0.368
Contrast sensitivity (log units)	0.109	**0.008**	0.019	0.778
Refractive error (diopters)	-0.082	**0.034**	-0.1	0.128
CSFT (μm)	-0.499	< **0.001**	-0.542	< **0.001**

Adjusted for age, duration of diabetes, visual acuity, colour vision, contrast sensitivity, CSFT, DR

5.4 Discussion

In this study, we investigated the structural variability of the fovea in terms of foveal slope in subjects with type II diabetes. Post hoc power analysis revealed 89.04 % power in estimating the difference in foveal slope between subjects with and without retinopathy. Foveal slope was found to be significantly shallow in subjects with STDR compared to other group of subjects in superior, inferior, and temporal quadrants. No difference in foveal slope was found in nasal quadrant. Foveal slope was found to be significantly shallow in older subjects with no retinopathy and those without STDR, and poor glycemic control was associated with steep foveal slope. Mean retinal thickness was significantly thicker in men compared to women in all the quadrants. However, we did not find any significant difference in foveal slope between men and women in all quadrants. Average foveal slope was significantly and negatively correlated with increase in age and CSFT after adjusting for other factors.

We found a trend of decreased foveal slope with increase in severity of DR in all the quadrants. However on Bonferroni correction, only subjects with STDR had a shallow inferior foveal slope compared to non-diabetic subjects and shallower superior, inferior, and temporal slope compared to those with no DR. This could be explained by the fact that as the foveal slope was negatively correlated with CSFT, subjects with STDR with more CSFT had a shallow foveal slope. Shallower slope also relates to the disproportional increase in retinal thickness in parafoveal and foveal areas in STDR. It may also be related to thinner subfoveal choroidal thickness in STDR, which was reported in the study conducted by Unsal et al.(101)

We found shallowing of foveal slope with increasing age in no DR and non-STDR groups. However, this age-related effect was not seen in STDR group. As it is known that the retinal thickness is increased in subjects with STDR, which includes cases with macular edema as well, in this group of subjects, the shallowing of foveal slope with increase in age might be compensated by increased retinal thickness.(25)

Our results are in agreement with those who reported reduced retinal thickness in women compared to men.(27,79,102) There have been reports on the effect of sex and race on foveal parameters such as foveal slope, foveal pit diameter and foveal pit depth, and reports that there is no gender-based difference in these parameters.(103) These data taken together support our finding that there is no gender-related difference in foveal slope.

In addition, we also assessed the correlation between the average foveal slope in the study sample and the ocular parameters. However, on adjusting for the variables using partial correlation, the factors that were found to be independently related to foveal slope were increase in age and CSFT. Foveal slope was significantly and negatively correlated with age. This can be

due to age-related neuronal loss.(104,105) Similarly, an increase in central macular thickness would shallow the slope.

This being a cross-sectional study, we could not see the changes in slope from no DR to non-sight-threatening DR to STDR. However, we got some interesting clue that could have clinical significance. We noted a trend of shallowing of foveal slope with increase in the severity of DR. Thus, sequential OCT with slope measurements may predict progression of DR. However, future longitudinal studies are required to validate these trends.

In conclusion, there are foveal slope changes specific for stages of DR, non-STDR, and STDR. Age and CSFT are independently related to slope. Further longitudinal studies are required to confirm the utility of these measurements.

Chapter 6

Chapter 6: Analysis of light and dark pixel density areas on SD-OCT in diabetes - Is it a marker for neuronal degeneration?

6.1 Introduction

Neuronal degeneration of retina is a critical component of DR. With the advanced diagnostic techniques like SD-OCT and fundus fluorescein angiography it is possible for the early diagnosis of DR and the management as well. Neuronal degeneration in diabetic retina takes place even before development of retinal lesions.(5,6) Various studies reported neuronal degeneration in terms of retinal thinning among subjects with diabetes but no retinopathy.(5,6,106) There are two hypotheses that account for the cells loss in the neural retina. First is due to the breakdown of blood-retinal barrier and other due to the direct effect of metabolism on the neural retina.(3)

The OCT signal from a particular tissue is based on the reflectivity and the absorption and scattering property of the underlying tissue. Relatively high reflective areas on OCT correspond to horizontal retinal elements such as RNFL, plexiform layers and RPE. Relatively low reflective area on OCT represents nuclear layers and PRL.(107) It is also reported that dark areas on the image represent homogenous material with low reflectivity such as air or clear fluids.(108) In this study we tried analyzing the SD-OCT images in terms of light and dark pixels, hypothesizing that increased dark pixels represents neuronal degeneration. This study results might pave way for the early diagnosis of neuronal damage in retina from SD-OCT images which being a non-invasive technique.

6.2 Methods

From the follow-up study (SN-DREAMS II), 801 eyes of 435 subjects with diabetes but no retinopathy were considered as cases and 58 eyes of 31 age matched subjects were considered as controls. SD-OCT retinal image processing and dark and light pixel calculations were done using Microsoft visual studio 6.0. All the "Dicom" format SD-OCT images were converted to "jpg" file format. Boundary of retina on SD-OCT images was automatically analyzed by software (Figure 6.2.1). Light area was considered if RGB value was more than 140 and dark area if RGB value was less than 140. The application software counts dark pixels and light pixels and also calculates light-dark (LD) ratio in a given SD-OCT image. As the acquired scan protocol contains 6 B-scans, the software calculates the average of light and dark pixel and LD ratio for all the scans. The results were further exported to Excel for review. The program was run on all the scans of SD-OCT of all the subjects. The software was set so as to proceed further only if at least 85% of the 7mm OCT line scan was clear enough for analysis.

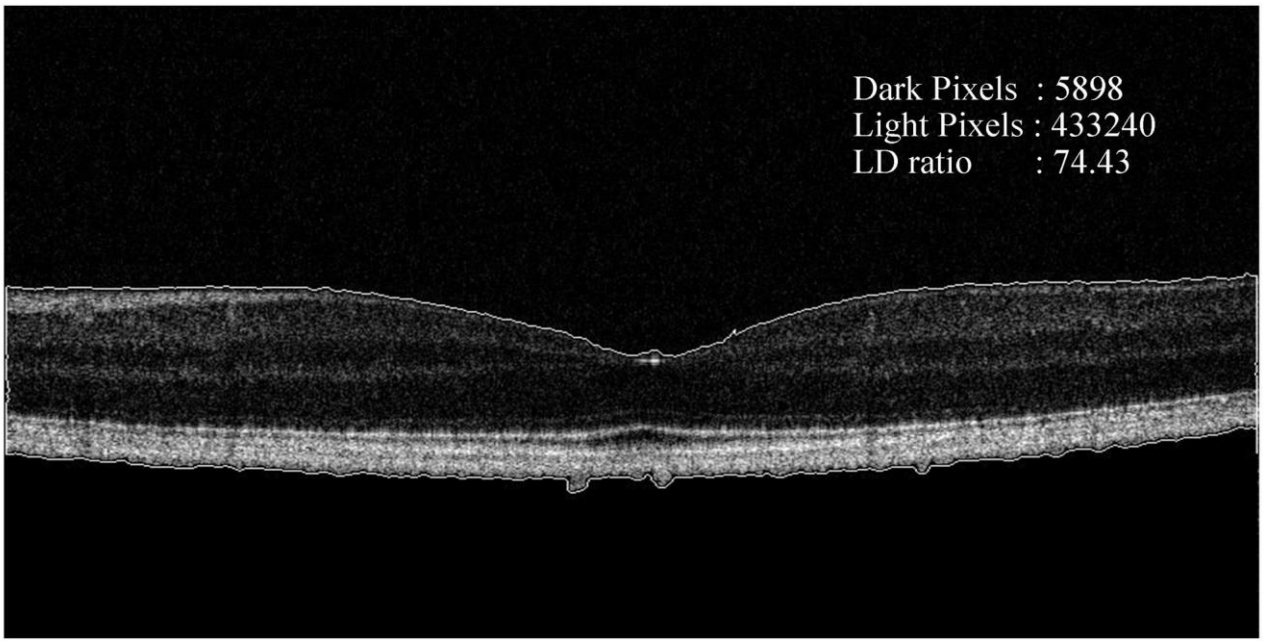


Figure 6.2.1: Result of the program showing automated detection of retinal boundary on SD-OCT cross sectional scan

6.3 Results

Total 482 eyes of 351 cases (subjects with diabetes but no retinopathy) and 33 eyes of 24 controls (non-diabetic subjects) were included for final analysis. Mean age of the study subjects was 57.45 ± 8.65 years and the mean duration of diabetes was 97.97 ± 72.17 months. Table 6.3.1 shows comparison of clinical parameters among the study groups. Dark pixel values were significantly high among cases (Cases: 8488.68 ± 3094.70, Controls: 6794.85 ± 1877.83; $p < 0.001$). No significant difference was found in the light pixel values and the LD ratio between the groups.

Table 6.3.1: Comparison of clinical parameters among the study groups

Parameters	Controls	Cases	*p*
Sample Size (eyes)	33	482	
Age (years)	54.61 ± 10.34	57.44 ± 8.61	0.072
Refractive error (spherical equivalent)	0.31 ± 1.14	0.02 ± 3.27	0.645
Dark Pixel	6794.85 ± 1877.83	8488.68 ± 3094.70	< **0.001**
Light Pixel	379702.40 ± 40586.22	395822.00 ± 51859.61	0.081
LD Ratio	60.49 ± 16.57	53.88 ± 21.77	0.088

Table 6.3.2 shows the correlation between the age and pixel parameters and we found no correlation exists between them.

Table 6.3.2: Correlation between age and pixel parameters

	R	*P*
Dark Pixel	0.054	0.222
Light Pixel	0.002	0.955
LD ratio	-0.074	0.095

Further we tried comparing the functional parameters among subjects with normal and abnormal dark pixels values. And for this we considered mean value of dark pixels among controls i.e., 6795 as a cutoff and we found that the sensitivity and specificity for this cutoff to be 67% and 52% respectively. On comparison of functional parameters between normal and abnormal dark pixel values (Table 6.3.3) we noted that none of the functional parameters were significantly different.

Table 6.3.3: Comparison of functional parameters among normal and abnormal dark pixel groups

	Normal n = 158	Abnormal n = 324	*P*
Age	56.56 ± 8.81	57.87 ± 8.50	0.116
Duration of diabetes	89.35 ± 53.79	90.85 ± 60.94	0.793
Visual acuity	0.06 ± 0.14	0.07 ± 0.14	0.332
Refractive error	0.19 ± 1.32	-0.06 ± 3.88	0.428
contrast sensitivity	1.37 ± 0.16	1.35 ± 0.18	0.366
TES	110.76 ± 54.08	128.58 ± 57.36	0.189
MRS	14.94 ± 2.80	14.29 ± 2.06	0.171

TES: Total Error Score; **MRS:** Mean Retinal Sensitivity

6.4 Discussion

In this study we tried analyzing SD-OCT images in terms of light and dark pixels using customized software. We found a significant increase in dark pixel values among subjects with diabetes but no retinopathy which indicate neuronal degeneration (involvement of nuclear layers) among these subjects when compared to non-diabetic subjects. Until recently, most research in terms of neuronal degeneration has been carried out on various functional tests (109) and on retinal thickness measurements.(5,6) Reflected light from the retinal layers gives information on the optical properties of tissue. So changes in layer intensity may provide complementary information regarding the effects of retinal disease.(110) To the best of our knowledge this is the first study to assess the optical property of the retinal tissue among subjects with diabetes but no retinopathy. Post hoc power analysis revealed 95.6% power in estimating the difference of dark pixel values between diabetic subjects without retinopathy and healthy individuals.

We did not find any difference in light pixel values and LD ratio between the groups. And also no correlation was noted between age and the pixel values. This suggests that the decrease in dark pixel values among these subjects is not related to age. However, studies have reported mixed results on correlation between retinal thickness and age. (111,112)

Dark pixel values were further classified as normal and abnormal based on the mean value of controls and compared the functional parameters among them. We found no significant difference in the functional parameters between the normal and abnormal pixel groups. This suggests that the neuronal changes found in terms of increased dark pixels are noted even before the functional vision is affected. However, studies have reported functional changes in terms of abnormal colour vision,(109) abnormal retinal sensitivity using microperimeter (5) and electroretinogram (11) among subjects with diabetes but no retinopathy suggesting that neuronal changes do happen even before microvascular changes in the retina.

There are few limitations to be considered when evaluating this study. First the sample of controls is very less when compared to cases and the sensitivity and specificity values for the cutoff value of dark pixel value is relatively less. Second, the shadowing of retinal blood vessels was not considered in the software, however this may not be an issue in the current study as the same protocol was followed for cases as well as controls. Third, we have used only 6 B-scans from asterisk scan protocol, information of pixel values from 3D scan protocol would have led to more precise information of the macula.

To summarize in this study we analyzed the dark and light pixel values in the SD-OCT images. Dark pixel values were increased among subjects with diabetes but no retinopathy suggestive of neuronal changes occurs even before change in the functional parameters.

Chapter 7

Chapter 7: Colour Vision Abnormalities in Type II Diabetes

7.1 Introduction

Despite effective treatment, DR remains the leading cause of legal blindness and moderate visual impairment among the working age group. (1,2) Various studies have reported that patients with diabetes exhibit acquired impairment of colour vision (ICV), the inability to discriminate hues.(46–48,113)

The FM 100-hue test is a widely used panel test for evaluating colour vision defects.(43,45) The moment of inertia method described by Vingrys et al(44) is a quantitative scoring method for panel tests. Previous studies, both clinic(47,48,113) and population-based(46) have shown an association between ICV and diabetes, with or without retinopathy. However, further quantification of the ICV was not done. Of the few epidemiological studies from India on DR, none have looked at ICV in subjects with diabetes.(66,114)

The aim of the present study was to assess colour vision abnormalities using the FM 100-hue test, quantifying it based on classical and moment of inertia methods, and to assess associated risk factors among subjects with type II diabetes in a population-based study.

7.2 Methods

Among the 867 subjects followed in the study, 673 eyes of 343 subjects had undergone the FM 100-hue test. Exclusion criteria included a BCVA worse than 6/12 and unwillingness or inability to understand and perform the FM 100-hue test. Subjects were said to have ICV if the TES based on the classical method fell outside the 95^{th} percentile for age, as published by Verriest et al,(45) for monocular testing without previous binocular experience and normal otherwise. Distribution of various ETDRS patterns of hue discrimination impairment was also analyzed.(115)

7.3 Results

The mean age of the study subjects was 57.16 ± 8.55 years (range: 44 – 86 years). The gender adjusted prevalence of ICV was 43% (CI: 39.2-46.7) among type II diabetics in the total sample. Gender adjusted prevalence of ICV among subjects with diabetes with no DR and those with DR was 42.7% (CI: 38.4- 47) and 43.4% (CI: 35.9 -50.9), respectively. Table 7.3.1 presents various risk factors for ICV among subjects with type II diabetes. Prevalence of ICV was significantly higher in men (54.4%) than in women (45.9%, $P = 0.017$). Systemic factors such as duration of diabetes, HbA1c, and systolic and diastolic blood pressure showed no association with prevalence of ICV. Significant ocular factors were reduced BCVA (log units) (0.06 ± 0.11 ICV Vs. 0.03 ± 0.09 no ICV; p = 0.003), the presence of PSC (3.6% ICV vs. 0.9% no ICV; *P*=0.034) and a history of cataract surgery (no cataract surgery ICV 89% vs. cataract surgery ICV 11%; *P*=0.012). The presence of DR was not associated with ICV, but the presence of CSME (5.7% ICV vs. 1.5% no ICV; *P*=0.003) and STDR (9.9% ICV vs. 4.6% no ICV; *P*=0.007) were significant factors associated with ICV.

Table 7.3.1: Risk factors associated with presence of colour vision impairment in type II diabetes

Risk factors	N =673	No ICV n(%)= 390 (57.9) n (%)	ICV n(%)= 283 (42.1) n (%)	*P*
Demographic & systemic Risk factors				
Gender				
Men	402	248 (63.8)	154 (54.4)	**0.017**
Women	271	142 (36.4)	129 (45.9)	
Duration of Diabetes (years)	-	9.69 ± 6.49	8.86 ± 5.61	0.141
HbA1c (%)	-	7.33 ± 1.79	7.55 ± 1.69	0.854
Systolic	-	132.67 ± 19.22	132.96 ± 20.11	0.804
Diastolic	-	77.42 ± 9.34	77.23 ± 10.46	0.081
Ocular Risk factors				
Visual Acuity (log MAR)	673	0.03 ± 0.09	0.06 ± 0.11	**0.003**
Cataract (LOCS III grade)				
None	511	289 (90.3)	222 (88.1)	0.394
Any	61	31 (9.7)	30 (11.9)	
Monotype				
NC	1	1 (0.3)	0(0.0)	1.000
CC	36	19 (5.9)	17 (6.7)	0.518
PSC	12	3 (0.9)	9 (3.6)	**0.034**
Mixed				
NC + CC	6	4 (1.3)	2 (0.8)	1.000
NC + PSC	2	0 (0.0)	2 (0.8)	1.000
CC + PSC	0			
NC+ CC + PSC	4	4 (1.3)	0 (0.0)	1.000
Cataract Surgery				
No	572	320 (82.1)	252 (89.0)	**0.012**
Yes	101	70 (17.9)	31 (11.0)	
Diabetic Retinopathy				
No DR	506	295 (75.9)	211 (74.6)	0.748
Mild DR	61	42 (10.8)	19 (6.7)	0.070
Moderate DR	60	35 (9.0)	25 (8.8)	0.950
Severe NPDR	7	5 (1.3)	2 (0.7)	0.705
PDR	17	7 (1.8)	10 (3.5)	0.156
Presence of CSME	22	6 (1.5)	16 (5.7)	**0.003**
Any DR	167	95 (24.4)	72 (25.4)	0.748
STDR	46	18 (4.6)	28 (9.9)	**0.007**

ICV: Impairment of colour vision; **NC:** Nuclear colour; **CC:** Cortical cataract; **PSC:** Posterior sub capsular cataract; **DR:** Diabetic Retinopathy; **NPDR:** Non-proliferative diabetic retinopathy; **PDR:** Proliferative diabetic retinopathy; **STDR:** Sight threatening diabetic retinopathy

Table 7.3.2 presents multiple logistic regression analysis of risk factors for presence of ICV in the study population. After adjusting for other significantly associated factors, it was shown that women were at higher risk of developing ICV (OR: 1.43, (1.04-1.98)). A history of cataract surgery was a protective factor (OR: 0.54, (0.34-0.86)). Subjects with CSME were 3 times more likely to have ICV. STDR remained a significant risk factor for ICV (OR: 1.98, (1.05-3.73)).

Figure 7.3.1 shows the distribution of 13 ETDRS patterns of hue discrimination impairment in our study sample. The most common patterns were C3 (28.7%), C2 (19%) and C1 (12.1%). Most of our subjects were found to have a blue-yellow colour defect.

Table 7.3.2: Multiple Logistic regression model for risk factors for impairment of colour vision

Risk factors	Adjusted	
	OR (95% of CI)	*P*
Gender		
Men	1	
Women	1.43 (1.04-1.98)	**0.028**
PSC*	3.35 (0.85-13.17)	0.083
Cataract Surgery	0.54 (0.34-0.86)	**0.009**
Diabetic Retinopathy		
No DR	1	
Mild DR	0.63 (0.35-1.21)	0.116
Moderate DR	0.99 (0.57-1.72)	0.960
Severe NPDR	0.46 (0.08-2.49)	0.365
PDR	1.84 (0.68-5.06)	0.239
Presence of CSME	3.07 (1.17-8.09)	**0.023**
STDR	1.98 (1.05-3.73)	**0.035**
Visual acuity (log MAR)	5.71 (0.88-36.99)	0.068

* Cataract surgery is not adjusted for PSC

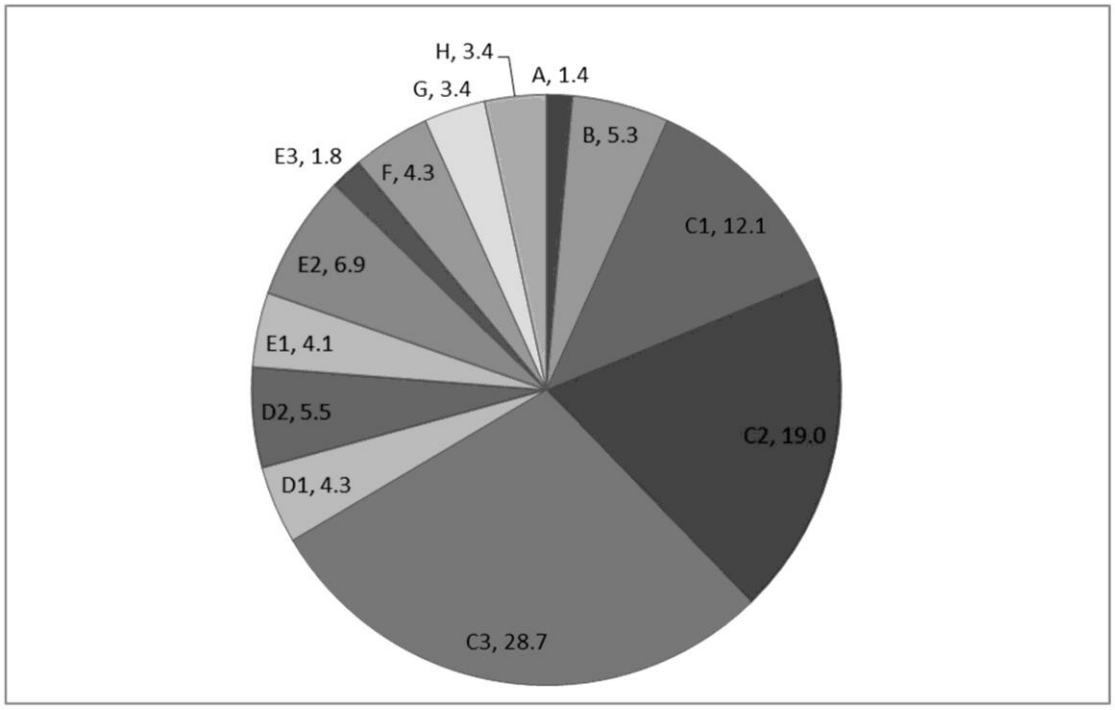


Figure 7.3.1: Distribution of ETDRS patterns of hue discrimination impairment

Table 7.3.3 shows colour vision characteristics based on the moment of inertia method in our sample. The angle of maximum radius was similar in all groups. Because of the random nature of cap placement our sample did not show any specific trend (protonope, deutranope, tritanope defects) of colour vision defects. There was a statistically significant difference in angle between normal and abnormal colour vision (69.7 ± 9.5 vs. 54.8 ± 3.9, *P*= 0.039) in severe NPDR. Both major and minor radii were high in those subjects with ICV in all subgroups; however, we did not observe polarity (S-index was not statistically significant between normal and abnormal ICV groups). But in subjects with no DR, there was a statistically significant difference in the S-index between the normal and abnormal colour vision groups (normal colour vision: 1.5 ±0.3 and abnormal colour vision: 1.6 ±0.3, p <0.05). Table 7.3.3 also shows a higher C-index in subjects with abnormal colour vision in all subgroups, suggesting a severe colour vision defect. This table also shows moment of inertia analysis for the C1, C2 and C3 ETDRS patterns of hue

discrimination impairment, and these 3 patterns showed increasing radii, S-index and C-index from C1 to C3.

Table 7.3.3: Colour vision characteristics based on moment of inertia method

Factors	CVI	N	Angle Mean ± SD	Major Radius Mean ± SD	Minor Radius Mean ± SD	TES Mean ± SD	Selectivity index Mean ± SD	Confusion index Mean ± SD
Over all subjects	Normal	390	62.4±12.4	5.4±1.4	3.5±0.7	6.4±1.5	1.5±0.3	2.1±0.5
	Abnormal	283	63.4±14.0	8.6±1.7**	5.5±1.4**	10.2±2.1**	1.6±0.3*	3.4±0.7**
No DR	Normal	295	61.5±12.8	5.3±1.4	3.5±0.7	6.4±1.5	1.5±0.3	2.1±0.6
	Abnormal	211	62.2±13.6	8.5±1.8**	5.4±1.4**	10.1±2.1**	1.6±0.3*	3.4±0.7**
Any DR	Normal	95	65.2±10.9	5.4±1.4	3.5±0.6	6.4±1.5	1.5±0.3	2.1±0.5
	Abnormal	72	66.9±14.9	8.6±1.6**	5.7±1.5**	10.4±2.0**	1.5±0.2	3.4±0.7**
Mild DR	Normal	42	63.3±10.6	5.3±1.4	3.5±0.7	6.3±1.4	1.5±0.2	2.1±0.5
	Abnormal	19	62.7±17.3	8.6±1.7**	5.6±1.3**	10.3±1.9**	1.5±0.2	3.3±0.8**
Moderate DR	Normal	35	63.9±11.7	5.4±1.6	3.4±0.62	6.5±1.6	1.6±0.3	2.2±0.6
	Abnormal	25	63.0±14.9	8.6±1.7**	5.7±1.9**	10.3±2.5**	1.6±0.2	3.4±0.7**
Severe NPDR	Normal	5	69.7±9.5	5.7±0.6	3.7±0.6	6.8±0.8	1.5±0.1	2.3±0.2
	Abnormal	2	54.8±3.9*	8.6±1.5	5.6±0.3*	10.2±1.4	1.5±0.2	3.4±0.6
PDR	Normal	7	73.5±6.3	5.2±0.4	3.4±0.2	6.2±0.4	1.5±0.1	2.0±0.2
	Abnormal	10	78.5±6.0	9.5±0.9**	6.1±0.7**	11.3±1.1**	1.6±0.2	3.8±0.6**
CSME	Normal	6	72.8±11.2	6.2±1.8	3.7±0.8	7.2±1.9	1.6±0.2	2.4±0.7
	Abnormal	16	72.3±11.1	8.3±1.5*	5.5±1.3*	10.0±1.9*	1.5±0.2	3.2±0.7
C1	Normal	89	64.9±12.5	4.8±0.9	3.2±0.5	5.8±1.0	1.5±0.4	1.9±0.8
	Abnormal	19	65.3±7.6	6.8±0.9**	4.4±0.5**	8.1±0.9**	1.5±0.2	2.7±0.4**
C2	Normal	81	64.9±12.9	5.6±1.2	3.6±0.6	6.6±1.3	1.5±0.2	2.2±0.5
	Abnormal	39	65.6±16.5	7.7±1.1**	4.9±0.6**	9.1±1.1**	1.6±0.2	3.0±0.4**
C3	Normal	74	61.9±13.9	6.4±1.3	4.0±0.7	7.6±1.3	1.6±0.3	2.5±0.6
	Abnormal	92	61.8±14.3	8.7±1.7**	5.4±1.2**	10.3±1.8**	1.6±0.3	3.4±0.7**

Statistical significance (* < 0.05, ** < 0.001)

7.4 Discussion

This population-based study found that the prevalence of ICV in subjects with type II diabetes was 42%; in subjects with diabetes but no retinopathy the prevalence of ICV was 41.6%; and in subjects with retinopathy, it was 43%. Various studies reported that patients with DR exhibit deterioration in the ability to discriminate hues.(47,116–117) Green et al(47) reported a lower prevalence of ICV in subjects with diabetes with no retinopathy (24%) and a higher prevalence in subjects with retinopathy (40%). Likewise, Trick et al(117) also reported the prevalence of ICV among diabetics with no retinopathy as 5.4% and among those with retinopathy as 10%. The difference in prevalence rates of ICV across other studies and our study can probably be attributed to differences in colour vision test methodology and analysis, as well as to differences in grading DR. Post hoc power analysis revealed 90% power in estimating the difference in the prevalence of ICV among subjects with and without retinopathy.

We found that women were at a higher risk of developing ICV than men. However, previous studies did not show this gender difference.(45–47,116) Eisner et al(118) showed evidence suggesting that the estrogenic response affects the colour naming of short-wavelength test stimuli presented on 580-nm backgrounds. Both types of estrogen receptor (ERα and ERβ) are present within the human retina;(119) but the roles of estrogen receptors for visual processing remain unknown.

Undergoing cataract surgery was a protective factor for ICV. With increasing age, yellow chromophores continuously accumulate inside the lens, reducing the transmission of blue light to the retina and resulting in blue-yellow colour vision defects. Normal age-related colour vision changes and those found in diabetic patients are predominantly seen in the blue-yellow colour

vision axis. Similar to our study, Ventruba(120) reported that colour vision was significantly improved in subjects who underwent cataract surgery.

Studies have reported that the incidence of abnormal colour discrimination correlates with the severity of retinopathy.(47) In our study STDR was found to be a significant risk factor for ICV. The presence of CSME was also a significant risk factor for ICV. Ventruba et al attributed this to a reduction in the transmission of light to the photoreceptors(120)

Subjects with type II diabetes showed both generalized errors of colour discrimination and specific patterns of colour vision defects. Even though the most commonly found ETDRS patterns were C1, C2 and C3, representing increasing severities of the blue-yellow defect, moment of inertia analysis failed to reveal any specific angle representing the tritan axis defect, and no polarity was revealed by the S-index. Because of the random nature of cap placement, our sample did not show any specific pattern defect; but the higher C-index in subjects with abnormal ICV in all subgroups suggests a severe colour vision defect. Also the patterns C1 to C3 represent an increasing severity of defect, as seen by the increasing C-index.

The limitation of our study being conducted in a specific diabetic population could not extrapolate the results to more general diabetic population as the sample size was calculated to estimate the prevalence of DR rather than for the ICV.

In summary, ICV was commonly observed among subjects with diabetes, both with and without retinopathy. Various risk factors for ICV were female gender and the presence CSME or STDR. A history of cataract surgery was a protective factor for ICV. The present study also shows that the ETDRS patterns C1-C3 represent increasing levels of colour vision defects, as confirmed by

the moment of inertia method. The high prevalence of ICV among diabetics suggests a possible need for occupational counseling.

Chapter 8

Chapter 8: Impairment of Colour Vision in Diabetes with no Retinopathy

8.1 Introduction

Various studies have demonstrated that individuals with DR can present with impairment of colour vision (ICV) and that the severity of ICV increases with increase in the severity of retinopathy and presence of DME.(46,113,123) It is reported that ICV was associated with DR, however the relationship between colour vision and DM with no retinopathy is not clearly defined.(124) Few studies have reported risk factors for ICV among subjects with diabetes.(46,49)

The aim of the present study was to report the prevalence of ICV among subjects with type II diabetes without any evidence of DR in a population based study and to assess various associated systemic and ocular risk factors for ICV.

8.2 Methods

Total 253 subjects with no clinical evidence of DR and who have undergone FM 100 hue-test were included for the current analysis. Subjects with BCVA worse than 6/12, uncooperative subjects and subjects who were unable to understand the test procedure were further excluded from the study. We also excluded those subjects with any congenital colour blindness, history of any ophthalmological disease and any chronic disease not associated with diabetes that could affect the visual system and also subjects who received any laser photocoagulation.

Colour discrimination was assessed monocularly with the FM 100-hue test and TES was assessed based on the classical method. We defined subjects with Impaired Colour Vision (ICV) based on the criteria that if the TES based on classical method fell outside the 95th percentile for

age as published by Verriest et al (45) for monocular testing without previous binocular experience and normal otherwise.

8.3 Results

The mean age of the study sample was 57.08 ± 9.21 (range: 44-86 years). Gender adjusted prevalence of ICV among subjects with diabetes having no retinopathy was 39.5% (CI: 33.5-45.5). Significant difference in the mean TES between ICV and no ICV groups was found (287.01 ± 81.04 vs 139.45 ± 60.32; $p< 0.001$). Table 8.3.1 presents various systemic risk factors for ICV among diabetic subjects with no retinopathy. There was a significant difference in age between ICV and no ICV groups ($p = 0.039$). The duration of diabetes was found to be high in the no ICV group (8.3 ± 5.4 years) compared to the ICV group (7.1 ± 3.9 years) with border line significance ($p = 0.054$) and resting heart rate was found to be significantly high in ICV group (77.9 ± 10.4) compared to no ICV group (73.7 ± 9.6) with $p = 0.001$. Biochemical parameters like HbA1c, serum total cholesterol, serum triglycerides, and serum low-density lipoprotein did not show any association with prevalence of ICV except for high-density lipoprotein which was significantly low in ICV (35.78 ± 8.8 in ICV vs 38.5 ± 11.1 mg/dl in no ICV; $p = 0.034$). Other parameters like presence of hypertension, mean ocular perfusion pressure, BMI, nephropathy and presence of neuropathy did not show any association with ICV.

Table 8.3.1: Systemic factors associated with impaired colour vision in diabetes without retinopathy

Risk factors	N =253	No ICV	ICV	*P*
		153	100	
Age (years)	253	58.0±9.4	55.6±8.8	**0.039**
Gender				
Men	149 (58.9)	97 (63.4)	52 (52.0)	0.072
Women	104	56 (36.6)	48 (48.0)	
Duration of Diabetes	253	8.3±5.4	7.1±3.9	0.054
HbA1c	253	7.2±1.8	7.4±1.8	0.501
Hemoglobin	253	13.9±2.1	13.9±1.7	0.987
Anemia				
Absent	206	121 (79.1)	85 (85.0)	0.237
Present	47	32 (20.9)	15 (15.0)	
Lipid Profile				
Serum total cholesterol	253	173.9±44.4	173.9±45.4	0.984
Serum HDL lipoprotein	253	38.5±11.1	35.78±8.8	**0.034**
Serum triglycerides	253	132.7±93.5	135.9±111.3	0.865
Serum LDL lipoprotein	253	104.7±35.2	104.2±41.3	0.918
Hypertension				
Absent	219	133 (86.9)	86 (86.0)	0.832
Present	34	20 (13.1)	14 (14.0)	
Resting heart rate	253	73.7±9.6	77.9±10.4	**0.001**
Mean ocular perfusion pressure	253	49.5±7.7	48.9±8.2	0.547
BMI (kg/m2)	253	25.6±5.7	25.7±7.5	0.935
Waist circumference	253	92.9±11.3	92.9±11.5	0.992
Nephropathy				
Normal	205	124 (81.0)	81 (81.0)	
Micro	41	25 (16.3)	16 (16.0)	0.982
Macro	7	4 (2.6)	3 (3.0)	
Neuropathy				
Absent	205	123 (80.4)	82 (82.0)	0.750
Present	48	30 (19.6)	18 (18.0)	

ICV: Impaired colour vision; **HbA1c:** Glycosylated hemoglobin; **BMI:** Body mass index; **HDL:** High density lipoprotein; **LDL:** Low density lipoprotein

Table 8.3.2 shows various ocular risk factors for ICV in subjects with diabetes without retinopathy. Significant ocular risk factors included subjects who had not undergone cataract surgery (no cataract surgery ICV 90 % Vs those who had previously undergone cataract surgery ICV 10%; $p = 0.023$) and IOP which was significantly high in the group with ICV (14.7 ± 3.1 in ICV Vs 13.9 ± 2.3 in no ICV; $p = 0.03$).

Table 8.3.2: Ocular risk factors associated with impaired colour vision

Risk factors	N =253	No ICV 153	ICV 100	*P*
Present of cataract (LOCS III grade)				
No Cataract	195	113 (93.4)	82 (91.1)	0.537
Any Cataract	16	8 (6.6)	8 (8.9)	
Monotype				
NC	1	1 (0.8)	0 (0)	1.000#
CC	8	5 (3.3)	3 (1.9)	1.000#
PSC	5	1 (0.8)	4 (4.4)	0.166#
Mixed				
NC + CC	2	1 (0.7)	1 (1.0)	1.000#
NC + PSC	-	-	-	
CC + PSC	-	-	-	
NC+ CC + PSC	-	-	-	
Cataract Surgery				
No	211	121 (79.1)	90 (90.0)	**0.023**
Yes	42	32 (20.9)	10 (10.0)	
Intraocular pressure	239	13.9±2.3	14.7±3.1	**0.03**
Contrast Sensitivity	223	1.3±0.21	1.3±0.17	0.996
OCT				
Central retinal thickness (1mm)	195	184.6±29.8	187.9±32.7	0.481
Central foveal thickness	223	170.6±22.6	171.3±19.5	0.824
Photoreceptor layer thickness	223	60.7±7.4	60.7±5.4	0.968
Mean retinal sensitivity	160	14.9±3.0	15.0±3.4	0.77

NC: Nuclear cataract; **CC:** Cortical cataract; **PSC:** Posterior sub capsular cataract; **OCT:** Optical coherence tomography; # Fishers exact test

Table 8.3.3 presents multiple logistic regression analysis of risk factors for presence of ICV in our study population. After adjusting for all other associated factors, it was shown that women were at higher risk of developing ICV (OR: 1.79, (1.00-3.18)). The duration of diabetes was no longer associated with ICV after adjusting for other factors. Increased IOP (OR: 1.12, (1.00-1.24)), resting heart rate (OR: 1.04, (1.01-1.07)) were significant risk factors.

Table 8.3.3: Multiple logistic regression model for risk factors for Impaired colour vision

Risk factors	ICV	
	OR (95% of CI)	*P*
Women	1.79 (1.00-3.18)	**0.049**
Duration of Diabetes	0.95 (0.89-1.02)	0.139
Anemia present	0.85 (0.41-1.77)	0.661
Serum HDL lipoprotein	0.96 (0.93-0.99)	**0.011**
HbA1c	0.99 (0.85-1.16)	0.926
Resting heart rate	1.04 (1.01-1.07)	**0.005**
Cataract Surgery done	0.46 (0.19-1.09)	0.079
Intraocular pressure	1.12 (1.00-1.24)	**0.048**

Adjusted variables: Gender, Duration of diabetes, Anemia, Serum HDL lipoprotein, HbA1c, Resting heart rate, cataract surgery, intraocular pressure

8.4 Discussion

Studies have found a relationship between ICV and DR (46,113,121) and also among subjects with diabetes but no retinopathy.(123) Prevalence of ICV as reported by Shoji et al (49) was 3.5 % in diabetic subjects without retinopathy. The prevalence of ICV in our study (39.5%) was higher compared to those reported earlier. Shoji et al (49) estimated ICV in two steps; Ishihara plates, a Lanthony 15-hue desaturated panel and Standard Pseudoisochromatic Plates Part 2 were

used to examine colour vision and FM 100-hue test was performed to define acquired colour vision impairment. Moreover, the study subjects were between 20-60 years, a predominantly younger population. This difference in the prevalence between our study and the other could be due to the difference in the study methodology and the sample population.. To the best of our knowledge this is the first study from India to report the prevalence of ICV in diabetic subjects without retinopathy.

Women were at higher risk of developing ICV in the study sample which was not found in the earlier studies.(45,46) It has been reported by Giuffre et al (124) that the performance of FM 100-hue test improved for 10 out of 15 participants at the time of ovulation, as opposed to during menstruation or at the beginning of the cycle. Eisner et al (118) have showed evidence suggesting that estrogenic response affects the colour naming of short-wavelength test stimuli presented on 580-nm background.

We observed higher prevalence of ICV among subjects who did not undergo cataract surgery, however it was not significantly associated with ICV in multiple logistic regression analysis. It is known that as the yellow chromophores accumulate inside the lens with increase in age it reduces the transmission of blue light to the retina which results in blue-yellow colour vision defects. Non-enzymatic glycation of lens proteins causes browning of the lens and that is known to be accelerated in diabetes.(125) Accelerated yellowing, rather than neuronal or vascular changes in the retina, thus appears to be the primary cause of colour vision anomaly observed in subjects with diabetes.(126) Ventruba J (120) reported that colour vision was significantly improved in subjects following cataract surgery which supports our results that undergoing cataract surgery is a protective factor.

Increased IOP was found to be a significant risk factor for ICV which was supported by previous studies.(127,128) This can be attributed to the fact that the short wavelength cones or their neuronal connections are less able to resist the raised IOP.(127) Further, the blue-yellow ganglion cells have a unique morphology and connectivity to second order neurons leading the blue-yellow ganglion cells to be more susceptible to IOP related damage.(128) The microvascular complications other than DR and neuropathy did not show any association with ICV in diabetic subjects.

Acquired ICV is an early indicator of neurodegenerative symptoms. It has been suggested that neural damage possibly precedes clinical DR. It has also been reported that the PRL thickness and retinal sensitivity were decreased in patients with diabetes, although no microvascular changes were noted in the retina.(5) On the contrary, in our study we did not find any further change in the SD-OCT thickness parameters and the mean retinal sensitivity of diabetic subjects having no retinopathy and with ICV. The evidence from this study supports the view that the ICV found in diabetic subjects without retinopathy may be of non-vascular etiology. Several hypotheses have been proposed such as osmotic distortion of the retina caused by fluid shifts inside the retina, followed by distortion and dysfunction of the neural cells and disorders of metabolism of neural cells caused by direct damage due to diabetes or because of the alterations of retinal microcirculation. The mechanism responsible for ICV is yet to be fully clarified and further research on these pathogenic mechanisms is needed.

To conclude, ICV was noted even among diabetic subjects without retinopathy. Significant risk factors for ICV were female gender and increased IOP. Our study finding re-emphasizes the importance of colour vision assessment even among subjects with diabetes but no retinopathy, which will be a useful method of screening and monitoring the diabetic subjects even without

retinopathy as colour vision plays an important role in subjects with diabetes to reliably self-monitor the blood and urine glucose levels.

Chapter 9

Chapter 9: Contrast Sensitivity and its Determinants in Diabetic Subjects

9.1 Introduction

Studies have shown that changes in the ganglion layer, inner retinal neurons, and peripheral network may be present in the asymptomatic stages of DR.(53) There is evidence of thinning of the inner retina in patients with minimal retinopathy.(129)

Various studies have evaluated CS in diabetes with and without DR.(50–52,54,130,131) Factors reportedly associated with abnormal CS in diabetes include age,(130) visual acuity,(51) duration of diabetes,(130) HbA1c(51) and presence of DR.(52,54) However, the reports are inconsistent. The relationship between abnormal CS and vascular and metabolic abnormalities in diabetes has not been extensively studied in the Indian population. The aim of the present study was to examine CS in a population-based cohort of subjects with type II diabetes and assess its correlation with systemic, biochemical, and ocular characteristics.

9.2 Methods

Total 653 subjects who have undergone CS assessment were included for the current analysis. One eye of each subject was analyzed. If the subject did not have DR in either eye, the right eye was selected; if DR changes were seen in one eye, that eye was included; and if both eyes had evidence of DR, the eye with more severe DR grade was considered for analysis.

9.3 Results

The mean age of the study sample was 58.7 ± 9.41 years (range: 44 to 87) and 384 of the subjects (58.8%) were male. Mean duration of DM was 8.94 ± 6.24 (0 to 37) years. Mean CS of the study sample was 1.32 ± 0.20 (0 to 1.65) log units. Table 9.3.1 shows the distribution of CS

among the demographic and systemic factors. CS was significantly reduced among the older age groups ($P < 0.001$). CS was noted to be significantly lower among subjects with a duration of diabetes of more than 5 years (1.30 ± 0.20 vs 1.36 ±0.19; $P < 0.001$), subjects with anemia (1.29 ± 0.20 vs 1.34 ± 0.20; $P = 0.002$), those with neuropathy (1.26 ± 0.21 vs 1.34 ± 0.19; $P < 0.001$), those who were taking insulin (1.27 ± 0.16 vs 1.33 ± 0.20; $P = 0.047$) and those with poor glycemic control ($P = 0.029$).

Table 9.3.1. Contrast sensitivity by demographic and systemic factors

Variables	N	Contrast Sensitivity (log units) Mean ± SD	*P*
Age 40 – 49	128	1.40 ± 0.19	
50 – 59	255	1.36 ± 0.17	**< 0.001**
60- 69	181	1.27 ± 0.21	
≥ 70	89	1.20 ± 0.20	
Gender			
Men	384	1.31 ± 0.20	0.256
Women	269	1.33 ± 0.20	
Duration of Diabetes			
≤ 5 (years)	250	1.36 ± 0.19	**< 0.001**
> 5 (years)	403	1.30 ± 0.20	
HbA1c (%)			
Normal (<5.6)	71	1.32 ± 0.17	
Good to fair (5.6 to 8.0)	402	1.34 ± 0.18	**0.029**
Poor (≥ 8.1)	180	1.29 ± 0.24	
Hypertension			
Absent	331	1.33 ± 0.19	0.108
Present	322	1.31 ± 0.20	
Anemia			
Absent	455	1.34 ± 0.20	**0.002**
Present	198	1.29 ± 0.20	
Nephropathy			
Absent	470	1.34 ± 0.18	0.051
Present	160	1.29 ± 0.25	
Neuropathy			
Absent	482	1.34 ± 0.19	**< 0.001**
Present	171	1.26 ± 0.21	
Serum total cholesterol (mg/dl)			
< 200	538	1.33 ± 0.20	0.246
≥ 200	115	1.30 ± 0.21	
Serum triglycerides (mg/dl)			
< 150	521	1.32 ± 0.20	0.772
≥ 150	132	1.32 ± 0.20	
Serum high-density lipoprotein (mg/dl)			
≥ 60	33	1.30 ± 0.25	0.348
<60	620	1.32 ± 0.20	
Serum low-density lipoprotein (mg/dl)			
< 100	318	1.33 ± 0.18	0.289
≥ 100	335	1.31 ± 0.21	
Treatment			
Non-Insulin	606	1.33 ± 0.20	**0.047**
Insulin	47	1.27 ± 0.16	

Table 9.3.2 shows the distribution of CS among the ocular parameters. CS was significantly reduced in those with BCVA worse than 6/6 (1.22 ± 0.22 vs 1.37 ± 0.17; $P < 0.001$), those with refractive error (1.30 ± 0.21 vs 1.35 ± 0.18; $P = 0.003$) and those with an abnormal colour vision; TES >100 (1.31 ± 0.19 vs 1.36 ± 0.14; $P = 0.047$). Other ocular factors associated with a significantly reduced CS were the presence of cataract ($P < 0.001$), presence of DR ($P = 0.003$) and presence of sight-threatening DR (1.20 ± 0.20 vs 1.30 ± 0.19; $P = 0.007$).

Table 9.3.2: Distribution of contrast sensitivity among various ocular factors

Variables	N	Contrast Sensitivity (log units) Mean ± SD	*P*
Best corrected visual acuity			
Normal (6/6 or less)	449	1.37 ± 0.17	**< 0.001**
Abnormal (more than 6/6)	202	1.22 ± 0.22	
Refractive error (spherical equivalent)			
Absent (-0.5 to +0.5 D)	236	1.35 ± 0.18	**0.003**
Present (< -0.50 and > 0.50)	417	1.30 ± 0.21	
Colour vision (FM 100- TES)			
≤ 100	51	1.36 ± 0.14	**0.047**
>100	144	1.31 ± 0.19	
Lens status			
No cataract	474	1.36 ± 0.18	
Any Cataract	84	1.19 ± 0.24	**< 0.001**
Pseudophakic	95	1.25 ± 0.20	
Cataract			
Nuclear cataract	8	1.14 ± 0.31	
Cortical cataract	42	1.26 ± 0.22	**0.028**
Posterior subcapsular cataract	11	1.17 ± 0.11	
Mixed	23	1.08 ± 0.24	
Diabetic Retinopathy			
No Diabetic Retinopathy	516	1.33 ± 0.20	
Mild NPDR	55	1.33 ± 0.20	
Moderate NPDR	47	1.27 ± 0.18	**0.003**
Severe NPDR	5	1.20 ± 0.18	
PDR	8	1.21 ± 0.15	
CSME	22	1.20 ± 0.23	
Any Diabetic Retinopathy			
Absent	516	1.33 ± 0.20	**0.003**
Present	137	1.28 ± 0.20	
Sight threatening diabetic retinopathy			
Absent	102	1.30 ± 0.19	**0.007**
Present	35	1.20 ± 0.20	
Central Subfield thickness (1mm area) (µm)			
≤ 190	261	1.35 ± 0.16	0.864
> 190	237	1.34 ± 0.19	
Photoreceptor layer thickness (µm)			
≤ 65.5	365	1.34 ± 0.18	0.953
> 65.5	174	1.34 ± 0.20	
Mean retinal sensitivity (dB)			
< 18.26	200	1.33 ± 0.21	0.052
≥ 18.26	14	1.45 ± 0.21	

Table 9.3.3 shows unadjusted analysis for the variables associated with CS. Factors associated with reduced CS included increased age (β= - 0.007, *P* <0.001), increased duration of diabetes (β = -0.004, *P* = 0.002), increased VPT score, i.e., diabetic neuropathy (β = -0.007, *P* <0.001), use of insulin (β = -0.06, *P* = 0.047), poor BCVA (β= -0.695, *P* <0.001), severity of DR (β = -0.029, *P*< 0.001), increased TES on the FM 100 (β = -0.001, *P* <0.001) and presence of cataract. An increase in mean retinal sensitivity (β = 0.021, *P* <0.001) and an increase in hemoglobin (β = 0.012, *P* = 0.002) were associated with increased CS.

Table 9.3.3: Univariate associations with contrast sensitivity

Risk factors	r value	Unadjusted		
		constant	β	*P*
Age (years)	-0.341	1.747	-0.007	< **0.001**
Duration of diabetes (years)	-0.122	1.357	-0.004	**0.002**
Glycosylated hemoglobin (HbA1c (%))	-0.068	1.384	-0.008	0.082
Hypertension	0.063	1.285	0.025	0.108
Hemoglobin (g/dl)	0.118	1.166	0.012	**0.002**
Vibration perception threshold (volts)	-0.238	1.448	-0.007	< **0.001**
Nephropathy	-0.092	1.379	-0.042	**0.021**
Serum Triglycerides (mg/dL)	-0.011	1.329	-0.006	0.773
Serum Total cholesterol (mg/dL)	-0.046	1.351	-0.024	0.246
Serum high density lipoproteins (mg/dL)	0.037	1.257	0.033	0.348
Serum low density lipoproteins (mg/dL)	-0.041	1.348	-0.017	0.291
Insulin intake	-0.078	1.387	-0.06	**0.047**
Best corrected visual acuity (log MAR)	-0.491	1.366	-0.695	< **0.001**
Spherical equivalent refractive error (decibels)	0.126	1.320	0.013	**0.001**
DR stages*	-0.161	1.365	-0.029	< **0.001**
Cataract				
NC	-0.208	1.553	-0.211	< **0.001**
CC	-0.232	1.494	-0.144	< **0.001**
PSC	-0.283	1.595	-0.247	< **0.001**
FM 100 TES	-0.311	1.424	-0.001	< **0.001**
Central subfield thickness (1 mm area) (µm)	-0.008	1.351	-0.003	0.864
Photoreceptor layer thickness (µm)	0.003	1.336	0.001	0.953
Mean retinal sensitivity (dB)	0.316	1.045	0.021	< **0.001**

* DR stages were stratified as no DR, Mild NPDR, Moderate NPDR, Severe NPDR, PDR, CSME

Table 9.3.4 summarizes the coefficients when analyzed in a multiple linear regression model and quantifies the relationship between CS and the variables. With a 0.1 log unit worsening of BCVA, CS is reduced by 0.563 units. With an increase in VPT score and presence of PSC, CS is decreased by 0.003 and 0.106 log units. We also found that a 1 grade increase in severity of DR was associated with an average of 0.016 units decrease in CS. With every 1 year increase in age, CS is reduced by 0.002 units.

Table 9.3.4: Multivariate linear association between contrast sensitivity and all variables

Risk factors	Adjusted				*P*
	Coefficient (β)	95% CI		Standard error	
		Lower bound	Upper bound		
Constant	1.699	1.577	1.821	0.062	< **0.001**
BCVA	-0.563	-0.689	-0.437	0.064	< **0.001**
VPT	-0.003	-0.006	-0.001	0.001	**0.006**
PSC	-0.106	-0.172	-0.041	0.033	**0.002**
Age	-0.002	-0.004	-0.001	0.001	**0.013**
DR stages*	-0.016	-0.029	-0.003	0.007	**0.015**

The variables adjusted are age, duration of diabetes, hemoglobin, VPT, Nephropathy, insulin intake, BCVA, DR stages, cataract status, FM 100 TES, mean retinal sensitivity, refractive error

* DR stages were stratified as no DR, Mild NPDR, Moderate NPDR, Severe NPDR, PDR, CSME

Stepwise linear regression was used for selection of variables for the multivariate model. Table 9.3.5 shows the 3 models to predict CS. Model 1 is based on the Systemic factors associated with CS, model 2 is for the prediction of CS based on ocular parameters and finally model 3 predicts the CS based on both systemic and ocular parameters. Our final model to predict CS was:

Predicted CS = 1.699 + (- 0.563) BCVA + (- 0.003) VPT score + (-0.106) presence of PSC + (- 0.002) age + (- 0.016) Stage of DR.

Table 9.3.5: Multivariate linear relationship models between contrast sensitivity and variables

R	R square	Adjusted R square	Std error of estimate
0.362 (a)	0.131	0.129	0.186
0.525 (b)	0.276	0.272	0.168
0.548 (c)	0.3	0.294	0.166

(a) Systemic predictors: (constant), Age, VPT

(b) Ocular predictors: (constant), BCVA, DR status, PSC

(c) All predictors: (constant), BCVA, VPT, PSC, Age, DR stages
VPT: Vibration perception threshold; BCVA: Best corrected visual acuity;
DR: Diabetic retinopathy

9.4 Discussion

In this population-based study we report that CS was significantly reduced in the presence of DR. The mean CS in our subjects with diabetes was 1.32 ± 0.20 log units, which was less than the 1.44 ± 0.05 log units reported by Stavrou et al.(50) The difference could be because they included only subjects with early DR, whereas our study included subjects with DR of various stages, as well as subjects with diabetes but without DR. Mantyjarvi et al(68) reported normal CS ranged from 1.68 to 1.84 log units in healthy individuals, which was high compared to our diabetic sample reported earlier.(51,52,130) We found an inverse association between CS and severity of DR consistent with previous reports.(52,54) However, these studies had several limitations, including the lack of adjustment of the various factors affecting CS, lack of demonstration of an established association among the risk factors and the CS. To the best of our knowledge this is the first study to assess various risk factors for CS in a population-based study in south India. The prevalence of DR in the study sample was 20.9%, and DME among subjects with DR was 16%. Prevalence of neuropathy was 26% and that of nephropathy was 25.39%.

Among the diabetic microangiopathies, DR and neuropathy were associated with CS, which was consistent with earlier reports.(50,52,54)

Age is an established risk factor for reduced CS (130) and this association was confirmed in our current study even in multiple linear regression analysis after adjusting for various other factors. Even though we found a significant negative correlation between CS and duration of DM, consistent with earlier study results,(54,130) our data showed no association on multiple linear regression. In contrast to other studies (51) we found no relationship between CS and HbA1c.

Anemia has been reported to be associated with the development and progression of both micro- and macrovascular complications of diabetes.(132) CS was positively correlated with hemoglobin levels in the present study. This may be due to the retinal vascular and visual defects caused by localized retinal tissue hypoxia in subjects with diabetes.(133) Studies have reported that hyperoxia significantly improved mean CS in subjects with diabetes.(134) VPT values showed a significant negative correlation with CS ($r = -0.238$, $P < 0.001$), which was in contrast to the study done by Moaven-Shahidi A et al,(135) who reported that CS did not vary between subjects with and without neuropathy. Srinivasan et al(136) in their study reported that the perifoveal thickness and RNFL thickness were inversely related to the severity of neuropathy.

A statistically significant inverse correlation was found between CS and log MAR visual acuity, which remained significant on multiple linear regression ($\beta=-0.563$, $P <0.001$), consistent with the results reported by Misra et al ($\beta= -0.427$, $P < 0.001$).(51) Even though TES of colour vision had a significant negative relation with CS, it did not show any further association with CS in multiple regression analysis.

Presence of PSC was significantly associated with reduced CS; consistent with earlier reports.(137,138) Studies reported that nuclear and cortical cataracts influence median and high frequency CS; whereas PSC impacted low-frequency CS.(139–141) Howes et al (137) reported that cataract has a more pronounced effect on CS function than DR with respect to the grating technique.

Mean retinal sensitivity was positively correlated with CS, but it showed no association on multiple regression analysis after adjusting for all variables. No correlation was found between CS and CRT or PRL thickness in this study. This could be explained by the early impact of diabetes on the inner retina,(23) even though outer retina is also involved. Both histopathologic and imaging studies have reported that diabetes causes apoptosis of ganglion cells and their dysfunction is primarily responsible for the abnormal CS.(23,129) Assessment of inner retinal thickness and function of ganglion cells would have added more value for this study.

Presence of DR was significantly associated with abnormal CS, as reported by previous studies using various techniques. Using an oscilloscope to generate gratings, Howes et al(137) found a systematic decrease in CS with severity of background DR. They also reported that background retinopathy produced abnormal CS at low and medium frequencies. In contrast, Sokol et al(54) reported that no specific spatial frequency is selectively affected in DR. With a 1-step increase in severity of DR, CS was reduced significantly by 0.016 units. The disturbance of visual function may be linked to vascular damage and thus correlated to the degree of retinopathy.

The regression model eventually obtained is presented in Table 9.3.5. This model 3 explains about 30% of the variation in CS. We also report that of the five variables from the final model, BCVA was more influential for the prediction of CS. Even though the r-square value is too low for the prediction of CS the main purpose of the models given in table 9.3.5 was to explore the

relative importance of ocular and systemic factors for the assessment of CS in type II diabetes sample. However, longitudinal studies are required to validate the model in clinical practice. Post hoc power analysis revealed 87.6% power in estimating the difference in contrast sensitivity values between subjects with and without retinopathy.

In conclusion, contrast sensitivity is impaired in DR. The changes in CS are related to both ocular and systemic characteristics in type II diabetes and not alone on severity of DR, as is clear from our study about various systemic and ocular risk factors associated with CS in subjects with diabetes.

Chapter 10

Chapter 10: Retinal Sensitivity in Subjects with Type II Diabetes Mellitus

10.1 Introduction

Visual acuity (VA) is the most widely used measure of macular function. However, in the early stages of the disease VA is not usually affected. Various functional tests like Humphrey visual fields, electro-retinogram (ERG), multifocal ERG and Microperimetry have demonstrated early damage in various retinal pathologies. Microperimeter (MP1) assesses the retinal sensitivity and fixation independently using an auto-eye-tracking system.

Evidence suggests that neuronal changes play an important role in the development of DR and that retinal degeneration may precede visible or vascular changes.(3,5) Studies have been reported assessing the correlation between the morphological changes using OCT and functional changes using MP1 in DME.(55,57,62,142) Sepah et al,(143) in their study reported a significant correlation between retinal thickness and sensitivity among patients with uveitis with and without macular edema. In our previous work we reported that mean retinal sensitivity (MRS) was significantly reduced with increase in the severity of DR and that it detects the early loss of retinal sensitivity in subjects with diabetes but no retinopathy.(10) To the best of our knowledge there is no population-based study assessing the functional changes among diabetes using MP1 and also studying various risk factors for impaired retinal sensitivity.

The aim of the current study was to assess retinal sensitivity among subjects with type II DM with and without DR in a population-based study and to assess risk factors for the impaired retinal sensitivity.

10.2 Methods

Among 867 subjects with type II diabetes followed in SN-DREAMS II, 357 subjects had undergone Microperimetry. Fifty eight age matched subjects with no DM and no ocular or retinal pathologies were included from our previous study on normative data of Microperimetry for comparison of MRS.(144) Eye with severe stage of DR for each subject was included for the final analysis.

Microperimetry was performed in the mydriatic state and MRS in the central 2°, 8°, 10°, 12°, and 20° was measured. Quadrant wise MRS was measured superonasal, superotemporal, inferonasal, inferotemporal to the fovea by selecting 5 stimuli in each quadrant (Figure 10.2.1). One single mean value was considered for retinal sensitivity in various degrees and quadrants. MRS that was calculated in 20° was defined as abnormal based on the age wise normative data of MRS that was established from our previous study in Indian population.(144) The normative values were established decade wise and the same values were used as cutoff for defining normal MRS in the present study.

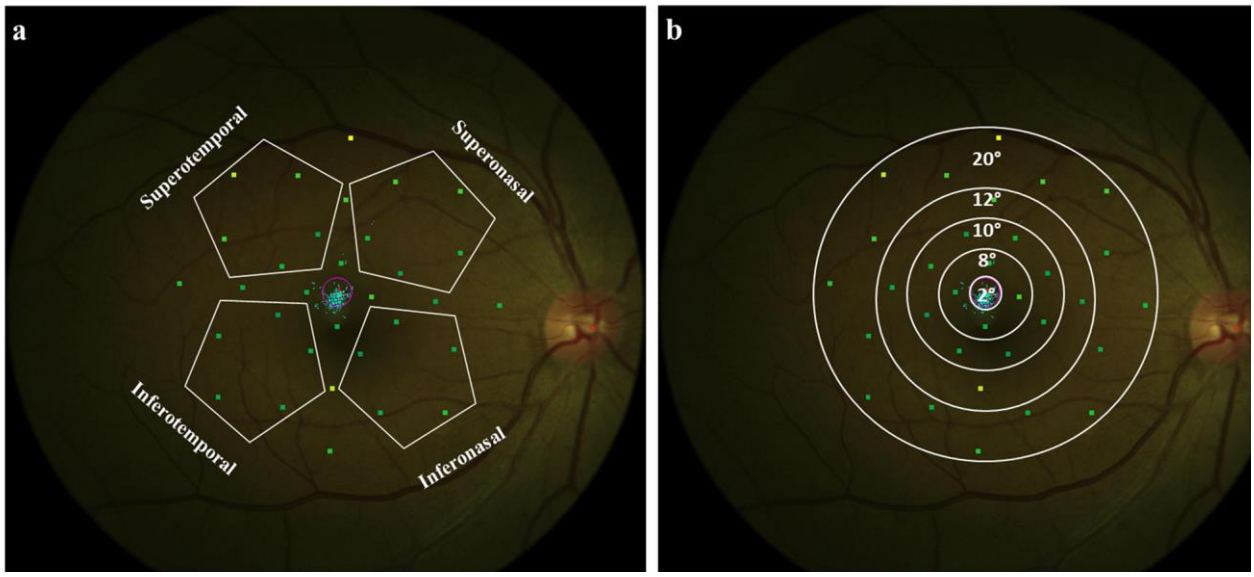


Figure 10.2.1: Schematic representation of MP1 Measures (a) Quadrant wise measurements (b) Degree wise measurements

CFT and PRL thickness in the central fovea were measured using SD-OCT. Mean retinal thickness in the central 1mm area was assessed. Morphological changes that were assessed on SD-OCT images are: Vitreoretinal interface which includes PVD and presence of ERM, altered foveal contour, abnormalities in the inner retinal layers which included presence of hard exudates and retinal thickening, altered PRL and RPE were based on the reflectivity and continuity of that particular layer. Figure 10.2.2 shows microperimetry grid overlapped on fundus image and cross-sectional image of SD-OCT.

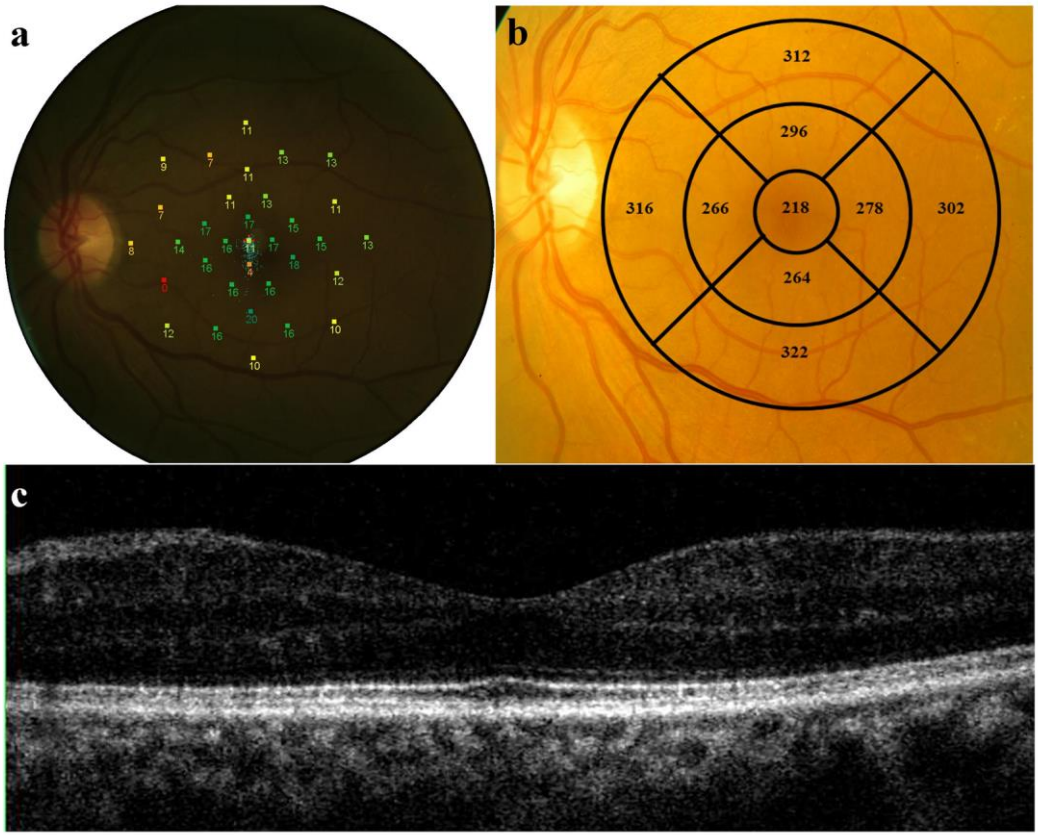


Figure 10.2.2: Shows (a) microperimetry test results, (b) ETDRS Thickness pattern of SD-OCT and (c) cross-sectional image of SD-OCT

10.3 Results

The mean age of the study subjects was 56.86 ± 8.63 years (range: 44 – 86). Mean spherical equivalent refractive error was 0.23 ± 5.18 diopters (range: -6.25 to 11.50). Mean duration of diabetes was 8.74 ± 5.81 years (range: 0 to 36 years). MRS was compared between no DR group and the published normative data of Microperimetry (age matched subjects with no DM).[15] MRS was significantly reduced in 2°, 8°, 12° and 20° in the no DR group compared to non-diabetic subjects. There was no significant difference in age between the subjects with different stages of DR (p=1.000). The increase in duration of diabetes was correlated with increase in

severity of retinopathy (p <0.001). The MRS was significantly reduced in moderate NPDR and ME at 8° and in ME at 10° and 12° when compared to subjects with diabetes but no retinopathy. The MRS was significantly reduced in superotemporal and inferotemporal quadrants in subjects with ME when compared to subjects with diabetes but no retinopathy. Moderate NPDR also showed significantly reduced MRS in inferotemporal quadrant (Moderate NPDR: 13.86 ± 3.82 vs no DR: 15.99 ± 3.20; p=0.021) (Table 10.3.1). The number of subjects with DR and more severity scale (Severe NPDR, PDR) were less in the study. Even though the differences between group 2 and other DR severity groups were statistically significant, clinically it may not be more relevant.

MRS was significantly reduced with increase in duration of diabetes (r= -0.139, *p*=0.008) and increased HbA1c (r= -0.112, *p*=0.035). Significant negative correlation was found between MRS and BCVA (log MAR) (r= -0.366, *p*<0.001). MRS was reduced with increase in CFT (r= -0.123, *p*= 0.025) and the 9 quadrants average retinal thickness (r= -0.125, *p*=0.033).

Table 10.3.1: Microperimetric characteristics in study population

	Non DM* (1)	No DR (2)	1 vs 2 p value	Mild NPDR (3)	2 vs 3 p value	Moderate NPDR (4)	2 vs 4 p value	Severe NPDR (5)	2 vs 5 p value	PDR (6)	2 vs 6 p value	ME (7)	2 vs 7 p value
n (%)	58	267 (74.8%)		36 (10.1%)	NA	29 (8.1%)	NA	4 (1.1%)	NA	7 (2%)	NA	14 (3.9%)	NA
Age (years)	54.22 ± 6.23	56.63 ± 8.94	0.052	57.47 ± 7.35	1.000	57.14 ± 9.39	1.000	59.75 ± 8.5	1.000	58.14 ± 3.23	1.000	57.64 ± 6.48	1.000
Duration of Diabetes (years)	NA	7.48 ± 4.59	NA	12.00 ± 7.40	**<0.001**	12.56 ± 8.26	**< 0.001**	11.75 ± 7.58	1.000	14.71 ± 3.35	**0.008**	12.59 ± 6.92	**0.01**
Spherical Equivalent refractive error (Diopter)	0.24 ± 0.82	0.23 ± 1.92	0.971	0.23 ± 1.26	1.000	0.32 ± 1.30	1.000	-0.78 ± 0.93	1.000	0.45 ± 0.77	1.000	0.26 ± 1.96	1.000
Retinal sensitivity (microns)													
2°	16.77 ± 2.04	12.60 ± 5.63	**<0.001**	13.19 ± 4.9	1.000	9.79 ± 6.13	0.191	10.5 ± 5.19	1.000	8.42 ± 7.74	0.875	10.5 ± 7.7	1.000
8°	18.19 ± 1.09	14.59 ± 3.84	**<0.001**	14.83 ± 3.19	1.000	12.27 ± 4.29	**0.04**	11.95 ± 1.97	1.000	11.68 ± 5.5	0.805	10.88 ± 5.57	**0.01**
10°	NA	15.16 ± 3.48	NA	15.34 ± 2.75	1.000	13.24 ± 3.81	0.086	12.85 ± 1.47	1.000	11.90 ± 4.89	0.242	11.81 ± 4.82	**0.009**
12°	18.15 ± 1.02	15.10 ± 3.44	**<0.001**	15.28 ± 2.75	1.000	13.28 ± 3.61	0.111	12.75 ± 1.79	1.000	12.10 ± 4.54	0.356	11.95 ± 4.69	**0.036**
Mean (20°)	17.68 ± 3.94	14.44 ± 3.38	**<0.001**	14.63 ± 2.89	1.000	12.71 ± 3.33	0.137	12.02 ± 2.59	1.000	11.69 ± 3.97	0.517	11.58 ± 4.53	**0.033**
Quadrant wise retinal sensitivity													
Superonasal	NA	13.19 ± 3.72	NA	13.96 ± 2.82	1.000	11.84 ± 3.37	0.296	11.10 ± 1.31	1.000	9.65 ± 3.94	0.166	11.04 ± 3.85	0.462
Superotemporal	NA	13.41 ± 3.61	NA	13.67 ± 2.47	1.000	11.83 ± 3.36	0.37	11.55 ± 3.54	1.000	10.00 ± 4.18	0.197	10.47 ± 5.02	**0.043**
Inferonasal	NA	15.52 ± 3.63	NA	15.53 ± 3.16	1.000	14.05 ± 3.53	0.598	13.10 ± 2.85	1.000	13.25 ± 4.7	1.000	12.62 ± 4.55	0.059
Inferotemporal	NA	15.99 ± 3.20	NA	15.92 ± 3.12	1.000	13.86 ± 3.82	**0.021**	12.65 ± 4.47	0.761	12.91 ± 4.63	0.271	13.15 ± 5.06	**0.035**

DM: Diabetes Mellitus; DR: Diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; ME: Macular edema

* Data of subjects with no DM was taken from our previous published study on normative data of Microperimeter

Quadrant wise retinal sensitivity among non DM was listed as NA as the data was not acquired in previous study

Table 10.3.2 shows univariate analysis of risk factors for abnormal MRS among subjects with type II diabetes. The prevalence of abnormal MRS in subjects with diabetes was 89.1%. Significant systemic parameters which were associated with abnormal MRS were duration of DM and HbA1c. Hypertension is not associated with abnormal MRS (p=0.543). Significant ocular parameters were BCVA (p=0.003) and contrast sensitivity (p=0.01). Presence of any DR, STDR and increased CFT were significantly associated with abnormal MRS.

Table 10.3.2: Risk factors associated with abnormal retinal sensitivity

Risk factors	Normal RS n(%) = 39 (10.9) n(%)/mean ± SD	Abnormal RS n(%) =318 (89.1) n(%)/mean ± SD	*P*	OR (95% of CI)	*P*
Demographic & systemic risk factors					
Age	53.69 ± 7.96	57.25 ± 8.64	**0.015**	1.06 (1.01-1.11)	**0.016**
Gender					
Men	22 (56.4)	185 (58.2)	0.833	1	
Women	17 (43.6)	133 (41.8)		0.93 (0.48-1.82)	0.833
Duration of Diabetes (years)(mean ± SD)	7.22 ± 4.07	8.93 ± 5.97	**0.024**	1.07 (0.99-1.15)	0.087
HbA1c	6.84 ± 1.36	7.46 ± 1.76	**0.012**	1.28 (1.02-1.61)	**0.034**
Hypertension					
Absent	22 (56.4)	163 (51.3)	0.543	1	
Present	17 (18.8)	155 (48.7)		1.23 (0.63-2.40)	0.543
Ocular risk factors					
Spherical equivalent refractive error (mean ± SD)	0.55 ± 2.10	0.19 ± 1.74	0.23	0.899 (0.76-1.07)	0.229
Best corrected visual acuity (log units)	0.01 ± 0.06	0.04 ± 0.11	**0.003**	1.17 (0.99-2.64)	0.054
Contrast sensitivity (log units)	1.42 ± 0.16	1.33 ± 0.19	**0.01**	0.045 (0.005-0.444)	**0.008**
Presence of cataract (LOCS III grade)					
None	34 (100)	246 (90.8)	0.65	NA	
Any	0	25 (9.2)			
Monotype					
Nuclear Cataract (NC)	0	2 (0.7)	0.615	NA	
Cortical Cataract (CC)	0	18 (6.6)	0.121	NA	
Posterior Sub-capsular Cataract (PSC)	0	6 (2.2)	0.381	NA	
Cataract Surgery					
No	34 (87.2)	271 (85.2)	0.743	1	
Yes	5 (12.8)	47 (14.8)		1.18 (0.44-3.17)	0.744
Intraocular pressure (mean ± SD)	14.63 ± 2.61	14.18 ± 2.39	0.29	0.93 (0.80-1.07)	0.294
Diabetic Retinopathy					
No DR	36 (92.3)	231 (72.6)	**0.008**	1	
Mild NPDR	3 (7.7)	33 (32.1)	0.782	1.71 (0.50-5.88)	0.392
Moderate NPDR	0	29 (9.1)	0.057	NA	
Severe NPDR	0	4 (1.3)	1.000	NA	
PDR	0	7 (2.2)	1.000	NA	
ME	0	14 (4.4)	0.308	NA	
Any DR	3 (7.7)	87 (27.4)	**0.008**	4.52 (1.36-15.05)	**0.014**
STDR	0	25 (7.9)	**0.06**	NA	
FM 100 TES (mean ± SD)	221.03 ± 90.17	207.44 ± 104.10	0.48	0.99 (0.99-1.00)	0.479
Central foveal thickness (mean ± SD)	163. 49 ± 16.69	173.14 ± 24.03	**0.021**	1.03 (1.01-1.04)	**0.017**
Mean retinal thickness (1mm) (mean ± SD)	184. 16 ± 15.56	195.24 ± 108.05	0.56	1.00 (0.99-1.02)	0.425
Mean retinal thickness (average of 9 quadrants)	264.78 ± 14.58	265.39 ± 27.62	0.84	1.00 (0.99-1.01)	0.901
Photoreceptor layer thickness (mean ± SD)	60.34 ± 5.48	60.76 ± 7.08	0.73	1.01 (0.96-1.07)	0.735

Table 10.3.3 summarizes the multivariate analysis which identifies independent risk factors related to abnormal MRS after adjusting for the variables which are significant from univariate analysis. Increased CFT remained a single significant risk factor for abnormal MRS with OR 1.02 (95% CI: 1.00-1.04) after adjusting for other factors.

Table 10.3.3: Multiple logistic regression model for risk factors for abnormal retinal sensitivity

Risk factors	Adjusted	
	OR (95% of CI)	*P*
Duration of diabetes	1.03 (0.95-1.13)	0.437
HbA1c	1.19(0.92-1.54)	0.178
Best corrected visual acuity	1.67 (0.92-3.04)	0.094
Contrast sensitivity (log units)	0.07 (0.01-1.10)	0.053
Any DR	2.24 (0.61-8.19)	0.222
Central foveal thickness	1.02 (1.00-1.04)	**0.044**

Table 10.3.4 shows MRS among various SD-OCT characteristics in the study sample. MRS was significantly reduced in subjects with altered foveal contour and those with presence of abnormalities in the inner retinal layers which includes presence of hard exudates or retinal thickening among subjects with DR. SD-OCT characteristics associated with abnormal MRS among subjects with diabetes but no retinopathy were presence of ERM, altered foveal contour and altered RPE. Presence of other characteristics did not show any change in MRS.

Table 10.3.4: Mean retinal sensitivity in various OCT characteristics

OCT characteristics	Subjects with Diabetic Retinopathy		*p*	Subjects with no Diabetic Retinopathy		*p*
	OCT done (n = 88)	Mean retinal sensitivity (dB)		OCT done (n = 249)	Mean retinal sensitivity (dB)	
Posterior vitreous detachment (PVD)	No PVD (75)	13.44 ± 3.34	0.310	No PVD (199)	14.54 ± 3.46	0.473
	Incomplete PVD (13)	12.39 ± 3.85		Incomplete PVD (50)	14.92 ± 2.60	
Epiretinal membrane	Absent (78)	13.38 ± 3.49	0.440	Absent (238)	14.74 ± 3.26	**0.008**
	Present (10)	12.50 ± 2.81		Present (11)	12.02 ± 3.28	
Foveal contour	Normal (80)	13.56 ± 3.35	**0.017**	Normal (244)	14.68 ± 3.28	**0.028**
	Altered (8)	10.55 ± 3.04		Altered (5)	11.42 ± 3.22	
Inner retinal layers	Normal (59)	13.98 ± 3.40	**0.006**	Normal (246)	14.65 ± 3.28	0.242
	Altered (29)	11.87 ± 3.03		Altered (3)	12.40 ± 5.50	
Photoreceptor layer	Normal (86)	13.30 ± 3.45	0.698	Normal (241)	14.69 ± 3.20	0.303
	Altered (2)	12.35 ± 1.62		Altered (8)	12.50 ± 5.55	
Retinal pigment epithelium	Normal (77)	13.34 ± 3.54	0.664	Normal (223)	14.78 ± 3.18	**0.030**
	Altered (11)	12.86 ± 2.43		Altered (26)	13.29 ± 4.08	

10.4 Discussion

The study reports a significant decline in MRS in subjects with diabetes but no retinopathy. However, the MRS was not much affected with varying severity of DR except for the moderate NPDR in central 8°. MRS was noted to be significantly reduced in subjects with ME. MRS in the temporal quadrant was significantly reduced in subjects with ME and moderate NPDR. Risk factor for abnormal MRS was increased CFT. MRS was significantly reduced among subjects with altered foveal contour and those with abnormalities in inner retinal layers. Post hoc power analysis revealed 83.72% power in estimating the difference of mean retinal sensitivity between subjects with and without retinopathy.

There was a statistically borderline difference in the age between the control groups and the diabetes group ($p = 0.052$). However, from our published normative data we report 0.04 dB per year age-related decline in MRS.(144) But the difference in MRS noted in subjects with diabetes but no retinopathy from our current study when compared to control group was higher which represents the effect of diabetes on retinal sensitivity rather than due to the age which was border line significant. This abnormal MRS in no DR group suggests the early neuronal changes that occur before the onset of vascular abnormalities.(5,6,109) We have earlier reported the structural changes in subjects with diabetes but no retinopathy using SD-OCT and reported that the CFT and the PRL thicknesses were significantly reduced in subjects with diabetes but no retinopathy compared to non-diabetic subjects.

Reduced retinal sensitivity was also reported in subjects with diabetes but no retinopathy.(5,6) Moderate NPDR also showed a reduction in MRS, probably due to preponderance of retinal lesions (micro aneurysms, hemorrhages, hard exudates and cotton wool spots) in macular area. Reduced MRS in the temporal quadrant was also reported earlier by Vujosevic et al in subjects with DME.(57) We did not find decreased MRS among subjects with severe NPDR and PDR, this could be explained by the fact that severe NPDR and PDR represent ischemic transformation in disease which primarily affects the retina beyond the arcades till the equator. The MRS gives the functional changes on the posterior pole this can be one of the reason why the retinal sensitivity changes does not mirror severity of retinopathy. It might also be due to very less sample in these groups.

MRS was reduced with increase in the duration of DM and HbA1C level. It has been reported that blood glucose and HbA1C values as an important predictor for the risk of onset of DR.(145) In addition HbA1C level is a known risk factor for severe visual loss.(146) The negative

correlation between HbA1C level and abnormal MRS in our study reinforces the importance of glycemic control in preventing visual loss in subjects with diabetes. MRS was significantly reduced with increase in the CFT and the average retinal thickness in 9 quadrants, however the correlations were weak. Earlier studies have reported similar negative correlation between MRS and retinal thickness in DME.(57)

Even though various parameters showed association with abnormal MRS, only increased CFT remained a significant risk factor on adjusting for others using multiple logistic regression analysis. Earlier studies reported reduced MRS with increase in retinal thickness in cases with DME.(57,142) Sepah et al also reported reduced retinal sensitivity with increase in retinal thickness in cases with uveitis.(143) A population based study done by Jansson et al among subjects with type I diabetes and retinopathy reported that retinal thickness was significantly associated with b-wave amplitude of photopic single-flash and 30-Hz flicker responses on full-field ERG.(147) Lobo et al (148) in their study have reported an increased retinal thickness and abnormal fluorescein leakage on angiography among subjects with diabetes but no retinopathy. They suggested that increase in retinal thickness was result of breakdown of blood-retinal barrier with extracellular deposition of macromolecules. Vujosevic et al (7) reported increased inner nuclear layer (INL) thickness among subjects with NPDR with no evidence of macular edema when compared to controls. They suggested that INL thickening may be due to the hypertrophy of Müller cells.

MRS was significantly reduced in subjects with DR with altered foveal contour and those with abnormalities in the inner retinal layers which included presence of hard exudates and increased retinal thickening which was supported by other studies.(55,56) This could be explained by the fact that hard exudates and thickening reduces the quality of image projected on the retinal

photoreceptors by blocking or scattering the light which suggests that the optical effects are major cause of abnormal MRS in these subjects. Among the subjects with diabetes but no retinopathy OCT characteristics leading to reduced MRS were presence of ERM, altered foveal contour and RPE alterations.

Three major findings of our current study are: (1) reduction in retinal sensitivity develops in a very high proportion of diabetes but no retinopathy which was never reported before, (2) reduced retinal sensitivity in subjects with diabetes but no retinopathy is a sign of early neuronal degeneration and (3) correlation between CFT and retinal sensitivity in subjects with DR, which may be important for microperimetry as a new functional biomarker in subjects with DME.

Earlier studies reported reduced amplitudes and delayed latencies of oscillatory potential in subjects with diabetes but no retinopathy using ERG.(8) According to the study done by Sokol et al (54) subjects with type II diabetes also had impaired contrast sensitivity, which again preceded vascular retinopathy but was worse in individuals with vascular changes. In our previous study we reported prevalence of colour vision impairment among subjects with diabetes but no retinopathy to be 39.5% and also reported that this might be due to the non-vascular etiology.(109) Combined with the results of earlier studies and our study which reports a high prevalence of reduced MRS among subjects with diabetes but no retinopathy suggest that these functional changes which include electrophysiological and psychophysiological measurements of retinal function might be due to the direct effect of diabetes on the neural retina, rather than secondary to the breakdown of the blood-retinal barrier. And alteration in foveal morphology may also play a part in these changes and cannot be attributed solely to neuronal changes.

Studies have reported that diabetes leads to early neuronal damage before the onset of vascular changes.(3,5) The elements that suggest chronic neuronal degeneration include neural apoptosis,

loss of ganglion cells, reduced inner retinal thickness, changes in electrophysiological activity and resultant deficits in perception.(3) Our study results of reduced MRS in diabetic subjects without DR added to those reported in literature suggests that chronic neuronal degeneration is an important aspect of vision loss in diabetic subjects even before development of retinopathy.

Few limitations of our study were that we have used a large grid with distant points (grid of 33 stimuli covering central 20 degrees) and further subdivision of MRS in 2, 8, 10 and 12 degrees would include only few tested points which can be a limit to the mean sensitivity data. And also consideration of individual point-wise retinal sensitivity and comparison with the corresponding retinal thickness values would have added value. The obtained results of our study may be referred just to the age group between 44 and 86 years as the decrease in retinal sensitivity is an age-related phenomenon this may limit the impact in real life. The numbers of DR with more severity scale (Severe NPDR, PDR) were less in the study. Even though the differences between group 2 and other DR severity groups were statistically significant, clinically it may not be more relevant. However, the trend warrants a validation study with an appropriate larger sample of severe forms of DR.

Reduced MRS occurs before morphological changes of DR indicating early neuronal damage in DM. Though there are trends of association of RS with glycemic control and BCVA, increase in the retinal thickness was the single most important risk factor for reduced RS with subjects with type II DM.

Chapter 11

Chapter 11: Fixation Characteristics among Subjects with Diabetes

11.1 Introduction

Visual acuity is used as a gold standard for functional vision assessment in various retinal diseases. It has been reported that VA is inadequate to quantify the visual function in patients with acquired macular disease as VA determination is unrelated to daily life activities.(149) Abnormal fixation stability is strongly associated with slower reading speed which is related to the patients vision related quality of life.(149) This ability to maintain steady fixation is impaired in patients with macular disorders.(150) Thus study of fixation is an important parameter to qualitatively assess visual function in patients with macular disorders. MP1 microperimetry is a reliable technique which assess the fixation characteristics in patients with macular diseases.(151)

In our previous study we reported significantly reduced mean retinal sensitivity and retinal thickness in subjects with diabetes without retinopathy and related it to neuronal damage.(5) However, we have not looked into the fixation characteristics among those subjects. Studies reported that the retinal sensitivity is reduced in patients with DME but the reports on fixation characteristics and DME are quiet contrasting.(55,57) Kube et al,(64) Carpineto et al(62) in their study reported that fixation stability was significantly affected in patients with DME. Vujosevic et al(65) reported that the fixation characteristics are independent of edema characteristics in patients with DME. Al Shafaee et al(152) in their study reported significant difference in the retinal sensitivity and fixation pattern in diabetic and prediabetic subjects when compared to controls. To the best of our knowledge there is no study assessing the fixation characteristics in subjects with diabetes with and without retinopathy in a population-based sample. Knowledge of

fixation characteristics might play an important role in assessing the quality of life even in subjects without DR. So, the aim of the current study was to analyze fixation characteristics in subjects with diabetes and with various stages of DR in a population-based study.

11.2 Methods

Among the 867 study subjects followed in the study, 357 subjects had undergone Microperimetry. One eye of subject, with more severe grade of retinopathy was included for the analysis e.g., if one eye of the subject had mild NPDR and other eye had PDR; the eye with PDR was included for the analysis.

MP1 Microperimetry was performed in the mydriatic state. Mean retinal sensitivity (MRS) was assessed. Fixation characteristics was measured according to Carpineto et al(62) and Fujii et al (Figure 11.2.1).(71) Scotoma was considered to be present if any of the stimulus point was recorded with 0 or <0 dB (Figure 11.2.2). If there was a marked asymmetry of VA between the 2 eyes, fixation stability may or may not be affected in the binocular testing condition. To overcome this we have performed MP1 monocularly by patching the other eye. Microperimetry characteristics were compared among the stable, incidence and progressed stages of DR compared to the baseline visit (SN-DREAMS I).

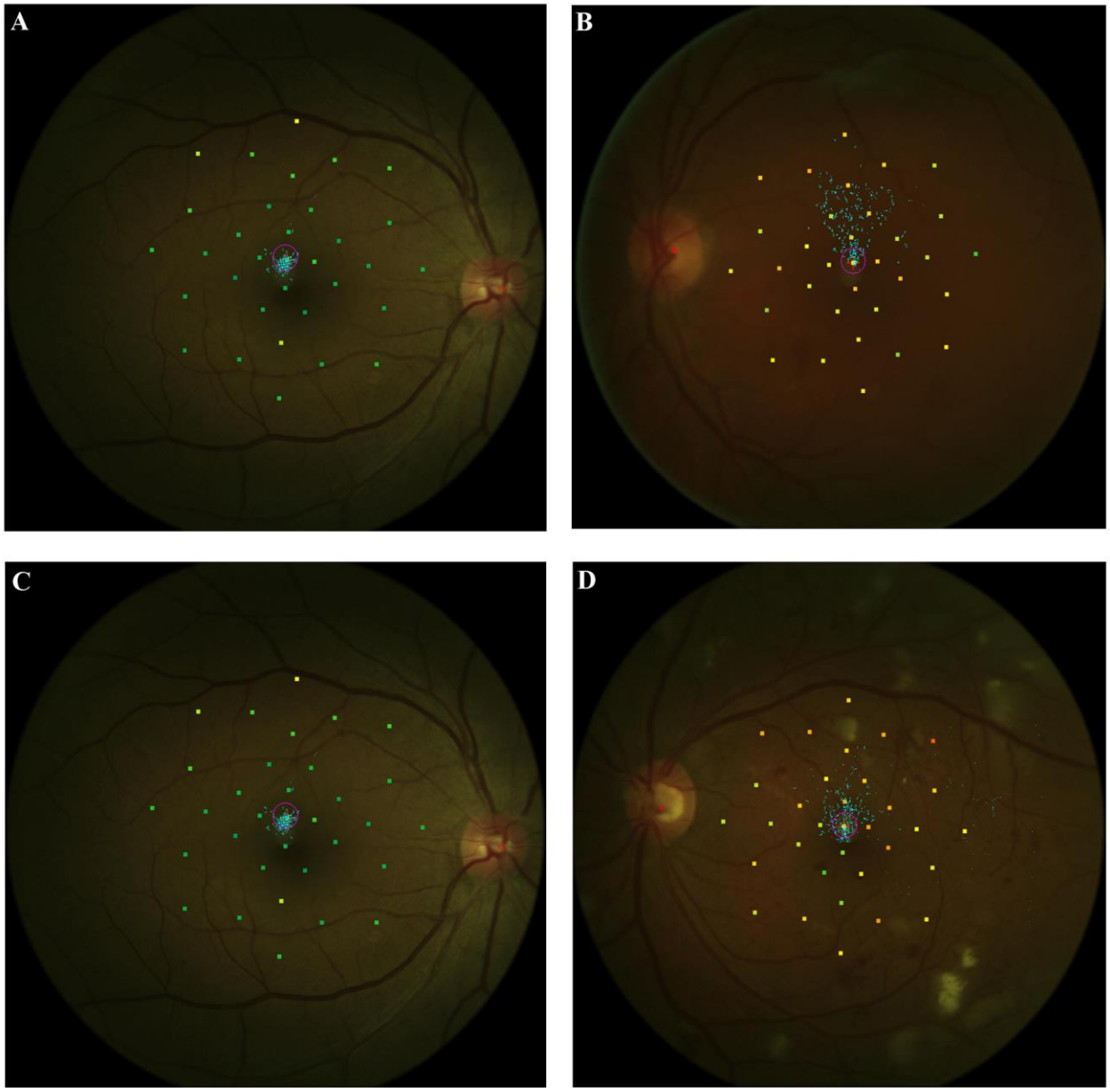


Figure 11.2.1: Microperimetric fixation characteristics among the study subjects: (A) Central fixation; (B) Poor central fixation; (C) Stable fixation; (D) Relatively unstable fixation

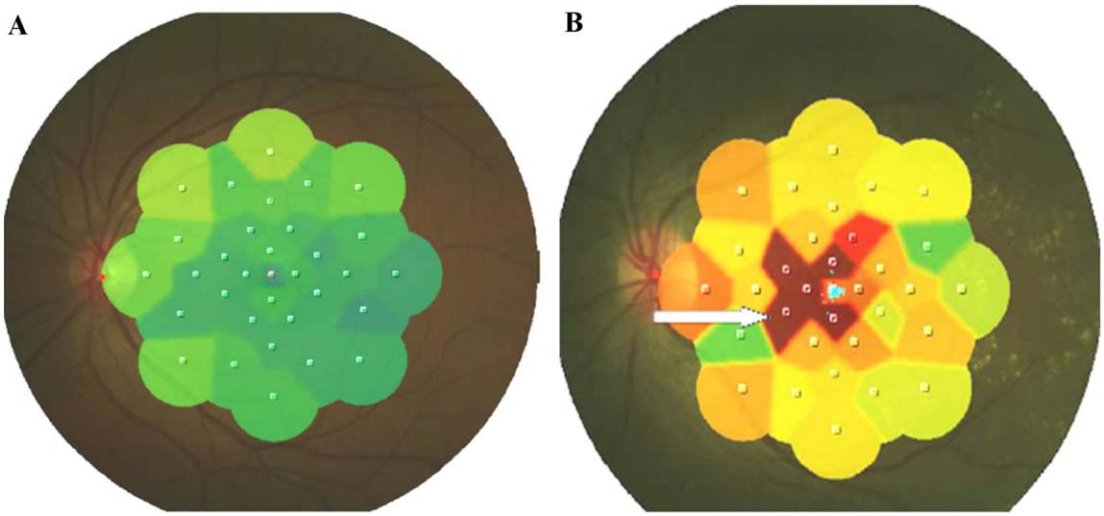


Figure 11.2.2: Microperimetry interpolated colour map showing (A) Normal (B) Presence of scotoma

SD-OCT thickness parameters which were analyzed are central foveal thickness, average retinal thickness in 9 ETDRS quadrants and PRL thickness. The following morphological changes were assessed on SD-OCT images: Vitreoretinal interface which included PVD and presence of epiretinal membrane (ERM), Abnormal foveal contour, abnormal inner retinal layers which included presence of hard exudates and retinal thickening, abnormal PRL and abnormal RPE.

11.3 Results

For the purpose of this study, eyes were further divided into 2 groups for fixation location and stability. Eyes with predominantly central fixation were included in central group and those with poor central and predominantly eccentric fixation were included in poor central fixation group. For the fixation stability, eyes with stable fixation were included in stable group and those with relatively unstable and unstable fixation were included in relatively unstable group similar to the study done by Carpineto et al.(62)

The mean age of the study subjects was 56.86 ± 8.63 years (range: 44 – 86) of which 58% were male. Mean duration of diabetes in the study population was 8.74 ± 5.81 years (range: 0 to 36 years). MRS in the study population was 14.13 ± 3.46 dB (range: 3.10 – 20 dB). Table 11.3.1 shows association between the study variables and fixation characteristics. It is observed that 73 subjects had relatively unstable and 25 subjects had poor central fixation among the diabetic subjects. Of the subjects with poor central fixation 72% (18 subjects) had relatively unstable fixation. Relatively unstable fixation was significantly associated with the location of fixation (central: 16.6 % vs poor central: 72%) with p <0.001. Distance BCVA was found to be significantly reduced in subjects with poor central fixation (central: 0.03 ± 0.09 vs poor central: 0.16 ± 0.18 log units; p= 0.002) and relatively unstable fixation (stable: 0.03 ± 0.09 vs relatively unstable: 0.07 ± 0.14; p= 0.017). Other variables did not show any relation with fixation characteristics.

Table 11.3.1: Association between variables and fixation characteristics

Parameters	Position of fixation		*P*	Stability of Fixation		*p*
	Central	Poor Central		Stable	Relatively Unstable	
No of eyes (%)	332 (93)	25 (7)		284 (79.6)	73 (20.4)	
Age	56.83 ± 8.74	57.36 ± 7.19	0.766	56.54 ± 8.17	58.14 ± 10.20	0.217
Gender						
Men	193 (58.1)	14 (56)	0.835	162 (57)	45 (61.6)	0.477
Women	139 (41.9)	11 (44)		122 (43)	28 (38.4)	
Duration of diabetes	8.81 ± 5.82	7.83 ± 5.75	0.417	8.68 ± 5.73	9.00 ± 6.14	0.667
HbA1C	7.42 ± 1.75	7.12 ± 1.45	0.412	7.37 ± 1.72	7.49 ± 1.78	0.600
Best Corrected Visual Acuity (log MAR)	0.03 ± 0.09	0.16 ± 0.18	**0.002**	0.03 ± 0.09	0.07 ± 0.14	**0.017**
Spherical Equivalent Refractive Error	0.24 ± 1.77	0.12 ± 2.00	0.756	0.24 ± 1.46	0.19 ± 2.72	0.883
Contrast sensitivity	1.34 ± 0.19	1.30 ± 0.15	0.303	1.34 ± 0.19	1.35 ± 0.15	0.561
Presence of cataract						
None	263 (79.2)	17 (68)	0.391	228 (80.3)	52 (71.2)	0.244
Any	22 (6.6)	3 (12)		18 (6.3)	7 (9.6)	
Cataract Surgery						
No	285 (85.8)	20 (80)	0.386	246 (86.6)	59 (80.8)	0.210
Yes	47 (14.2)	5 (20)		38 (13.4)	14 (19.2)	
Retinal sensitivity						
2°	12.21 ± 5.84	12.60 ± 5.22	0.752	12.36 ± 5.86	11.76 ± 5.55	0.429
8°	14.20 ± 4.04	14.12 ± 3.90	0.922	14.28 ± 3.99	13.86 ± 4.17	0.433
10°	14.82 ± 3.62	14.52 ± 3.70	0.689	14.93 ± 3.56	14.31 ± 3.81	0.195
12°	14.78 ± 3.54	14.52 ± 3.67	0.715	14.89 ± 3.48	14.28 ± 3.78	0.192
Mean	14.14 ± 3.45	13.97 ± 3.63	0.811	14.22 ± 3.39	13.76 ± 3.70	0.305
Diabetic Retinopathy						
No DR	247 (74.4)	20 (80)	0.534	210 (73.9)	57 (78.1)	0.468
Mild NPDR	34 (10.2)	2 (8)	1.00	32 (11.3)	4 (5.5)	0.143
Moderate NPDR	27 (8.1)	2 (8)	1.00	21 (7.4)	8 (11)	0.320
Severe NPDR	4 (1.2)	0	1.00	4 (1.4)	0	0.586
PDR	7 (2.1)	0	1.00	7 (2.5)	0	0.352
Macular edema	13 (3.9)	1 (4)	1.00	10 (3.5)	4 (5.5)	0.497
Any DR	85 (25.6)	5 (20)	0.534	74 (26)	16 (21.9)	0.468
STDR	24 (7.2)	1 (4)	1.00	21 (7.4)	4 (5.5)	0.567
Central foveal thickness	171.75 ± 22.43	177.13 ± 35.19	0.281	170.94 ± 22.81	176.72 ± 25.84	0.069
Central subfield thickness	194.17 ± 105.46	192.60 ± 36.80	0.947	195.14 ± 113.40	189.72 ± 29.31	0.714
Mean retinal thickness	265. 44 ± 26.64	263.73 ± 24.63	0.787	265.65 ± 27.65	264.07 ± 21.45	0.684
Photoreceptor layer thickness	60.80 ± 7.02	59.67 ± 5.49	0.441	60.68 ± 7.22	60.84 ± 5.69	0.868

Prevalence of scotoma was found to be 24.4 % among the study sample. Table 11.3.2 shows clinical characteristics of the study subjects with and without scotoma. Women had high prevalence of scotoma than men (51.7 % vs 48.3 %) with $p = 0.035$. BCVA and the contrast sensitivity were found to be significantly reduced in subjects with scotoma ($p < 0.001$). Prevalence of scotoma was significantly related with presence of any cataract (19.5% vs 3 %; $p < 0.001$). Scotoma was significantly associated with CC and PSC. MRS was significantly reduced in subjects with scotoma in central 2, 8, 10, 12 and 20 degrees ($p < 0.001$ respectively). We did not find any relation between presence of scotoma and fixation characteristics. Presence of scotoma was related with severe NPDR ($p = 0.046$), macular edema ($p = 0.008$) and STDR ($p < 0.001$). Retinal thickness did not show any relation with presence of scotoma in the study sample.

Table 11.3.2: Clinical characteristics influencing prevalence of scotoma

Parameters	Scotoma		*P*
	Present (n= 87)	Absent (n =270)	
Age	58.26 ± 9.12	56.41 ± 8.44	0.082
Gender Men	42 (48.3)	165 (61)	**0.035**
Women	45 (51.7)	105 (39)	
Duration of diabetes	9.02 ± 5.53	8.65 ± 5.91	0.607
HbA1C	7.53 ± 2.08	7.35 ± 1.60	0.388
BCVA	0.096 ± 0.15	0.023 ± 0.07	**<0.001**
Spherical equivalent Refractive error	0.004 ± 2.19	0.30 ± 1.64	0.240
Contrast sensitivity	1.24 ± 0.18	1.37 ± 0.18	**<0.001**
Cataract status			
No cataract	57 (65.5)	223 (82.6)	< 0.001
NC	1 (1.1)	0	0.243
CC	10 (11.5)	7 (2.6)	**0.002**
PSC	5 (5.8)	1 (0.4)	**0.004**
Mixed	1 (1.1)	0	0.243
Cataract Surgery			
No	74 (85)	231 (85.6)	0.909
Yes	13 (15)	39 (14.4)	
Retinal sensitivity			
2°	6.11 ± 5.39	14.22 ± 4.36	**<0.001**
8°	9.69 ± 4.03	15.64 ± 2.75	**<0.001**
10°	10.65 ± 3.68	16.14 ± 2.37	**<0.001**
12°	10.75 ± 3.67	16.06 ± 2.33	**<0.001**
Mean	10.30 ± 3.59	15.36 ± 2.34	**<0.001**
Stability of Fixation			
Stable	64 (73.6)	220 (81.5)	0.111
Relatively unstable	23 (26.4)	50 (18.5)	
Location of fixation			
Central	78 (89.7)	254 (94)	0.160
Poor central	9 (10.3)	16 (6)	
Diabetic Retinopathy			
No DR	60 (69)	207 (76.7)	0.150
Mild DR	3 (3.4)	33 (12.2)	**0.018**
Moderate DR	9 (10.3)	20 (7.4)	0.383
Severe NPDR	3 (3.4)	1 (0.4)	**0.046**
PDR	4 (4.6)	3 (1.1)	0.063
Macular edema	8 (9.2)	6 (2.2)	**0.008**
Any DR	27 (31)	63 (23.3)	0.150
STDR	15 (17.2)	10 (3.7)	**<0.001**
Central foveal thickness	177.13 ± 32.87	170.60 ± 19.64	0.097
Central subfield thickness	195.33 ± 38.17	193.73 ± 113.62	0.912
Mean retinal thickness	269.08 ± 29.60	264.35 ± 25.58	0.218
Photoreceptor layer thickness	59.84 ± 7.17	60.98 ± 6.83	0.212

Fixation and scotoma characteristics in relation to SD-OCT characteristics among subjects with DR were presented in table 11.3.3. No relationship was found between fixation characteristics and various DR lesions on SD-OCT. Prevalence of scotoma was significantly high in subjects with abnormal foveal contour (19.2 % vs 4.8%; $p = 0.046$) and abnormal inner retinal layers (61.5 % vs 22.6%; $p < 0.001$).

Table 11.3.3: Fixation parameters in various OCT characteristics of diabetic retinopathy

OCT Characteristics		Fixation Location		*P*	Fixation Stability		*p*	Scotoma		*p*
		Central (83)	Poor Central (5)		Stable (72)	Relatively unstable (16)		Present (26)	Absent (62)	
Posterior vitreous detachment (PVD)	No PVD (75)	70 (84.3)	5 (100)	1.000	60 (83.3)	15 (93.8)	0.448	21 (80.8)	54 (87)	0.515
	IPVD (13)	13 (15.7)	0		12 (16.7)	1 (6.3)		5 (19.2)	8 (13)	
Epiretinal membrane	Absent (78)	73 (88)	5 (100)	1.000	64 (90)	14 (87.5)	1.000	23 (88.5)	55 (88.7)	1.000
	Present (10)	10 (12)	0		8 (11)	2 (12.5)		3 (11.5)	7 (11.3)	
Foveal contour	Normal (80)	76 (91.6)	4 (80)	0.386	65 (90.3)	15 (93.8)	1.000	21 (80.8)	59 (95.2)	**0.046**
	Abnormal (8)	7 (8.4)	1 (20)		7 (9.7)	1 (6.3)		5 (19.2)	3 (4.8)	
Inner retinal layers	Normal (58)	55 (66.3)	3 (60)	1.000	49 (68)	9 (56.3)	0.393	10 (38.5)	48 (77.4)	< **0.001**
	Abnormal (30)	28 (33.7)	2 (40)		23 (32)	7 (43.8)		16 (61.5)	14 (22.6)	
Photoreceptor layer	Normal (86)	81 (97.6)	5 (100)	1.000	70 (97.2)	16 (100)	1.000	25 (96.2)	61 (98.4)	0.506
	Abnormal (2)	2 (2.4)	0		2 (2.8)	0		1 (3.8)	1 (1.6)	
Retinal pigment epithelium	Normal (77)	73 (88)	4 (80)	0.496	63 (87.5)	14 (87.5)	1.000	22 (84.6)	55 (88.7)	0.725
	Abnormal (11)	10 (12)	1 (20)		9 (12.5)	2 (12.5)		4 (15.4)	7 (11.3)	

Of 357 subjects, 10.6% (38 subjects) had newly developed DR, 7.6% (27 subjects) had further progression of DR compared to baseline and 82% (292 subjects) were stable compared to the baseline (SN-DREAMS I). Microperimetric characteristics were compared in these 3 groups and found that the MRS was significantly different in central 8, 10, 12 and 20 degrees of the macula (Table 11.3.4). On further analysis using Bonferroni we found significant difference only between stable and progressed group of subjects 8° (p = 0.027), 10° (p = 0.02), 12° (p = 0.029) and 20° (p = 0.04). We did not find any significant difference in fixation characteristics between the groups. Prevalence of scotoma was significantly high in progressed group followed by stable and incidence groups (p = 0.023).

Table 11.3.4: Microperimetric characteristics in progression and incidence cases of Diabetic retinopathy

MP Characteristics	Diabetic Retinopathy			*P*
	Stable Cases	Incidence Cases	Progressed Cases	
n (%)	292 (82)	38 (10.6)	27 (7.6)	
Retinal sensitivity				
2°	12.22 ± 5.73	13.71 ± 5.58	10.44 ± 6.47	0.08
8°	14.32 ± 3.99	14.63 ± 3.72	12.21 ± 4.42	**0.026**
10°	14.94 ± 3.61	15.01 ± 3.17	12.97 ±3.95	**0.023**
12°	14.90 ± 3.55	14.90 ± 3.08	13.06 ± 3.80	**0.034**
Mean (20°)	14.27 ± 3.47	14.14 ± 3.12	12.55 ± 3.50	**0.046**
Fixation Location				
Central	270 (92.5)	36 (94.7)	26 (96.3)	0.686
Eccentric	22 (7.5)	2 (5.3)	1 (3.7)	
Fixation Stability				
Stable	230 (79)	34 (89.5)	20 (74)	0.234
Unstable	62 (21)	4 (10.5)	7 (26)	
Scotoma				
Present	69 (23.6)	6 (15.8)	12 (44.4)	**0.023**
Absent	223 (76.4)	32 (84.2)	15 (55.6)	

Stable: No change in the retinal during follow-up compared to baseline visit; **Incidence:** Had no DR at baseline but developed retinopathy at follow-up; **Progression:** Had some DR at baseline and increased in severity in follow-up

11.4 Discussion

In the current population-based study 20.4% of the study subjects had relatively unstable fixation and 7% had poor central fixation. Our results indicate that the VA was significantly reduced in subjects with relatively unstable and poor central fixation. Even though statistically significant difference in BCVA was noted between the stable and relatively unstable fixation groups the difference in VA was not clinically significant. And the stability of fixation was significantly associated with location of fixation i.e. majority of subjects with poor central fixation had relatively unstable fixation. Fixation stability and fixation location were not related to any other variables including SD-OCT characteristics. Our results were in contrast to those reported by Carpineto et al(62) who concluded that VA, central retinal sensitivity, foveal thickness, duration of symptoms, HbA1c levels, history of PRP, and presence of cystoid changes were strongly associated with fixation impairment in subjects with CSME. This difference in results could be explained by the fact that the earlier studies included subjects with more severe stages of retinopathy, whereas ours being a population-based study mostly included subjects with early stages of retinopathy and subjects with no DR as well. Vujosevic et al(65) evaluated retinal fixation characteristics in patients with DME and reported that fixation location and stability are independent of edema characteristics which supports our study results that both fixation location and stability were not influenced by presence of CSME.

Among those subjects with diabetes and poor central fixation, 80% did not have any DR, and, among those with relatively unstable fixation 78% did not have any DR. Our findings were supported by Al Shafaee et al(152) who reported abnormal fixation patterns in 40% of the subjects with diabetes without retinopathy and suggested that these finding might support the hypothesis that neuronal degeneration precedes macroangiopathy.

The prevalence of scotoma was found to be 24% in subjects with type II diabetes in the current study. Our results indicate that presence of scotoma was significantly associated with reduced VA, female gender and reduced contrast sensitivity. It has been reported that the contrast sensitivity was lower in subjects with diabetes and it is further reduced with increase in the severity of DR.(52,54,134) Harris et al(134) in their study reported that isocapnic hyperoxia improved contrast sensitivity in subjects with diabetes with no or early DR, suggesting that the tissue hypoxia initiates both visual and vascular dysfunction.

Cataract was significantly related to the presence of scotoma which was supported by Richter-Mueksch et al(153) who reported that existence and specification of cataract highly influence central retinal sensitivity. Varga et al in their study reported that density of posterior capsule opacification (PCO) was significantly correlated with BCVA and macular sensitivity.(154) This could be explained by the fact that density of cataract reduces the amount of light entering into the eye and the quality of image projected on the retinal photoreceptors leading to abnormal retinal sensitivity in these subjects. In subjects with scotoma the MRS in central 2, 8, 10, 12 and 20 degrees was significantly reduced. Probably the cataract affecting the visual axis may reduce sensitivity in 1 or more locations to less than or equal to 0 dB among the 33 point stimulated, which would come under our classification of scotoma.

Prevalence of scotoma was significantly associated with severe NPDR, PDR, macular edema and subjects with STDR. In subjects with mild NPDR the prevalence of scotoma was found to be significantly less. This could be supported by the reason that the DR lesions involved in the macula of mild NPDR are less compared to those in severe stages which could lead to reduced MRS leading to presence of scotoma. Prevalence of scotoma was significantly associated with abnormal foveal contour and abnormal inner retinal layers which included hard exudates and

diffuse retinal thickening. Mori et al(61) in their study reported prevalence of scotoma to be 74% among subjects with CSME and presence of scotoma causes unstable fixation and impaired VA. We did not find any relation between central scotoma and fixation characteristics which could be explained by the fact that scotoma was considered to be present if any one stimulus point was recoded with retinal sensitivity of 0 or < 0 dB. Location of scotoma might be in the foveal or parafoveal area in central 20 degrees which might not impair the fixation stability of the subjects. Post hoc power analysis revealed 90.18% power in estimating the difference in prevalence of scotoma among subjects with and without retinopathy.

We found significantly reduced MRS and higher prevalence of scotoma among the progressed group of retinopathy. From this study we suggest that even though subjects with diabetes with and without DR have impaired retinal sensitivity and fixation characteristics, further progression of DR will lead to further impairment of MRS and increased prevalence of scotoma.

As earlier studies reported, bivariate contour ellipse area (BCEA) measurement provides a better quantification of fixation stability.(155) However, when current study was performed, the initial version of MP1 software was used, which did not had an option of BCEA calculation. Hence the data points were not available for analysis which might be the limitation of our study.

To summarize, we report the prevalence of fixation instability and poor central fixation along with presence of scotoma among diabetics with and without DR in a population-based study. Abnormal fixation characteristics were associated with reduced VA. Presence of scotoma was significantly high in women, among subjects with cataract, abnormal contrast sensitivity, abnormal retinal sensitivity and presence of STDR. Prevalence of scotoma and further impairment of MRS was significantly associated with progression of DR.

Chapter 12

Chapter 12: Incidence, Progression, and Associated Risk Factors of Posterior Vitreous Detachment in Type II Diabetes Mellitus

12.1 Introduction

Vitreoretinal interface and vitreous detachment have an important role in the progression of DR. The vitreous changes in both clinical (34) and autopsy studies (35) have shown that liquefaction and syneresis of vitreous are more common among people with diabetes. Also, the higher incidence and early onset of posterior vitreous detachment (PVD) has been reported among subjects with diabetes even without DR.(35,36) In our previous study,(38) we reported the prevalence of PVD to be 63.3% among the subjects with diabetes. Risk factors for PVD were increasing age, male gender, presence of sight-threatening DR, and increased axial length.

There are evidences that progression of no PVD to CPVD (CPVD) had a protective effect on the development of PDR (42); as with CPVD, the scaffold for vascular proliferation gets removed. However, there is lack of a population-based follow-up study to show the incidence and progression of PVD, their risk factors, and their influence on DR.

The aim of this study was to report the incidence and progression of PVD and factors influencing the same in a cohort of patients with type II diabetes in South Indian population.

12.2 Methods

Of the 867 study subjects who were followed up for 4 years, PVD status was available for 615 subjects. Eye with more severe grade of DR was included for the analysis. The status of PVD was detected by indirect ophthalmoscopy and confirmed by B-scan ultrasonography (Ultrascan; Alcon Laboratories, Sinking Spring, PA) and SD-OCT.

Definitions

Refractive Error

- Emmetropia was defined as a spherical equivalent refractive error between –0.50 and +0.50 D.
- Myopia was defined as spherical equivalent error less than –0.50 D.
- Hyperopia was defined as spherical equivalent error greater than +0.50 D.

Follow-up Status of PVD

- **Stable PVD:** When there is no change in the status of PVD compared to the baseline visit.
- **Conversion to IPVD:** Status of PVD being converted to IPVD in the follow-up from no PVD at baseline visit.
- **Conversion to CPVD:** Status of PVD being converted to CPVD in the follow-up from no PVD or IPVD at baseline visit.

PVD characteristics were compared among the improved, stable, incidence, and progressed stages of DR compared to the baseline visit (SN-DREAMS I).

- **Improvement:** Presence of some stage of DR at baseline, a one-step or more decrease in severity of retinopathy at follow-up.
- **Stable:** No change in the status of DR at follow-up compared to baseline visit.
- **Incidence of DR:** Those who had no DR at baseline but developed DR during follow-up.
- **Progression of DR:** Those who had some stage of DR at baseline and further increase in severity of DR by one step or more during follow-up.

12.3 Results

The mean age of the study subjects was 59.12±9.44 years (range: 44–87); 51.5% were men. The mean duration of diabetes was 9.06±6.19 years (range: 0–37). Table 12.3.1 shows the distribution of PVD stages at baseline and follow-up visits. Of the subjects with no PVD at baseline, 80.8% developed IPVD or CPVD and 32.63% progressed to CPVD from IPVD in the baseline visit.

Table 12.3.1: Incidence of posterior vitreous detachment

		Follow-up Status		
		No PVD (n)	IPVD (n)	CPVD (n)
Baseline Status	No PVD	51	168	47
	IPVD	0	192	93
	CPVD	0	0	64

PVD: Posterior Vitreous detachment; **IPVD:** Incomplete PVD, **CPVD:** Complete PVD

Table 12.3.2 shows the characteristics of stable PVDs. It was noted that the age was significantly high in subjects with stable IPVD and stable CPVD compared to that in subjects with no PVD (*P* = 0.001 and <0.001, respectively). High prevalence of emmetropia was observed among subjects with stable no PVD (stable no PVD: 52.9% vs stable IPVD: 37.5%; *P* = 0.046) (stable no PVD: 52.9% vs stable CPVD: 21.9%; *P* = 0.001). Subjects with stable CPVD had increased duration of diabetes (stable no PVD: 8.19±4.91 vs stable CPVD: 11.80±6.48; *P* = 0.001), high prevalence of myopia (stable no PVD: 21.6% vs stable CPVD: 48.4%; *P* = 0.003), and increased axial length

(stable no PVD: 22.75±1.10 vs stable CPVD: 23.57±1.40; *P* = 0.001) compared to those with stable no PVD.

Table 12.3.2: Characteristics of stable posterior vitreous detachment

	Stable No PVD (n = 51)	Stable IPVD (n = 192)	*P*	Stable CPVD (n = 64)	*p*
Age	53.94 ± 7.45	58.54 ± 9.14	**0.001**	66.80 ± 8.76	< **0.001**
Gender					
Male	27 (52.9)	104 (54.2)	0.876	45 (70.3)	0.056
Female	24 (47.1)	88 (45.8)		19 (29.7)	
Duration of diabetes	8.19 ± 4.91	8.92 ± 6.19	0.436	11.80 ± 6.48	**0.001**
Diabetic retinopathy					
No DR	37 (72.5)	145 (75.5)	0.664	47 (73.4)	0.915
Non STDR	10 (19.6)	33 (17.2)	0.687	12 (18.8)	0.907
STDR	4 (7.8)	14 (7.3)	1.000	5 (7.8)	0.995
DR Status Compared to baseline					
Improved	1 (2)	4 (2.1)	1.000	2 (3.1)	1.000
Stable	42 (82.4)	157 (81.8)	0.924	53 (82.8)	0.948
Incidence	3 (5.9)	16 (8.3)	0.771	7 (10.9)	0.508
Progressed	5 (9.8)	15 (7.8)	0.579	2 (3.1)	0.238
Refractive error					
Emmetropia	27 (52.9)	72 (37.5)	**0.046**	14 (21.9)	**0.001**
Myopia	11 (21.6)	46 (24)	0.720	31 (48.4)	**0.003**
Hyperopia	13 (25.5)	74 (38.5)	0.084	19 (29.7)	0.618
Axial length	22.75 ± 1.10	22.79 ± 1.08	0.822	23.57 ± 1.40	**0.001**
Cataract surgery					
Not done	50 (98)	180 (93.8)	0.312	59 (92.2)	0.225
Done	1 (2)	12 (6.3)		5 (7.8)	

Table 12.3.3 summarizes the characteristics of unstable no PVD, that is, conversion of no PVD at baseline to IPVD and CPVD at follow-up visit. Prevalence of hyperopia was more in subjects converted to IPVD from no PVD compared to those with stable no PVD (stable no PVD: 25.5% vs conversion to IPVD: 42.9%; *P*=0.026). Factors associated with conversion of no PVD to

CVPD are increase in age (stable no PVD: 53.94±7.45 vs conversion to CPVD: 58.79±8.39; *P*=0.003), myopia (stable no PVD: 21.6% vs conversion to CPVD: 40.4%; *P*=0.043), and undergoing cataract surgery (stable no PVD: 2% vs conversion to CPVD: 17%; *P*=0.013).

Table 12.3.3: Characteristics of unstable no posterior vitreous detachment

	Stable No PVD (n = 51)	Conversion to IPVD (n = 168)	*P*	Conversion to CPVD (n = 47)	*P*
Age	53.94 ± 7.45	56.07 ± 7.90	0.089	58.79 ± 8.39	**0.003**
Gender					
Male	27 (52.9)	70 (41.7)	0.156	20 (42.6)	0.304
Female	24 (47.1)	98 (58.3)		27 (57.4)	
Duration of diabetes	8.19 ± 4.91	8.18 ± 5.47	0.987	7.62 ± 5.00	0.570
Diabetic retinopathy					
No DR	37 (72.5)	140 (83.3)	0.087	39 (83)	0.216
Non STDR	10 (19.6)	20 (11.9)	0.161	6 (12.8)	0.360
STDR	4 (7.8)	8 (4.8)	0.481	2 (4.3)	0.459
DR Status compared to baseline					
Improved	1 (2)	5 (3)	1.000	0	1.000
Stable	42 (82.4)	143 (85.1)	0.633	44 (93.6)	0.125
Incidence	3 (5.9)	10 (6)	1.000	3 (6.4)	1.000
Progressed	5 (9.8)	10 (6)	0.349	0	0.057
Refractive error					
Emmetropia	27 (52.9)	69 (41.1)	0.135	15 (31.9)	**0.036**
Myopia	11 (21.6)	27 (16.1)	0.364	19 (40.4)	**0.043**
Hyperopia	13 (25.5)	72 (42.9)	**0.026**	13 (27.7)	0.808
Axial length	22.75 ± 1.10	22.80 ± 1.02	0.805	23.05 ± 1.30	0.223
Cataract surgery					
Not done	50 (98)	160 (95.2)	0.689	39 (83)	**0.013**
Done	1 (2)	8 (4.8)		8 (17)	

Conversion to IPVD & CPVD from no PVD at baseline

Factors associated with conversion of IPVD to CPVD are increase in age (stable IPVD: 58.54±9.14 vs conversion to CPVD: 63.55±9.87; $P < 0.001$), presence of myopia (stable IPVD: 24% vs conversion to CPVD: 36.6%; $P = 0.026$), high axial length (stable IPVD: 22.79±1.08 vs conversion to CPVD: 23.12±1.13; *P*=0.021), and undergoing cataract surgery (stable IPVD: 6.3% vs conversion to CPVD: 14 %; $P = 0.031$). Factors responsible for remaining stable IPVD

are presence of no DR (stable IPVD: 75.5% vs conversion to CPVD: 49.7%; $P < 0.001$) and emmetropia (stable IPVD: 37.5% vs conversion to CPVD: 21.5%; $P = 0.007$) (Table 12.3.4).

Table 12.3.4: Characteristics of unstable incomplete posterior vitreous detachment

	Stable IPVD (n = 192)	Conversion to CPVD (n = 93)	*P*
Age	58.54 ± 9.14	63.55 ± 9.87	**< 0.001**
Gender			
Male	104 (54.2)	51 (54.8)	0.915
Female	88 (45.8)	42 (45.2)	
Duration of diabetes	8.92 ± 6.19	10.24 ± 7.60	0.150
Diabetic retinopathy			
No DR	145 (75.5)	71 (49.7)	**< 0.001**
Non STDR	33 (17.2)	16 (17.2)	0.997
STDR	14 (7.3)	6 (6.5)	0.795
DR Status Compared to baseline			
Improved	4 (2.1)	1 (1.1)	1.000
Stable	157 (81.8)	78 (83.9)	0.662
Incidence	16 (8.3)	7 (7.5)	0.815
Progressed	15 (7.8)	7 (7.5)	0.932
Refractive error			
Emmetropia	72 (37.5)	20 (21.5)	**0.007**
Myopia	46 (24)	34 (36.6)	**0.026**
Hyperopia	74 (38.5)	39 (41.9)	0.583
Axial length	22.79 ± 1.08	23.12 ± 1.13	**0.021**
Cataract surgery			
Not done	180 (93.8)	80 (86)	**0.031**
Done	12 (6.3)	13 (14)	

Conversion to CPVD from IPVD at baseline

Table 12.3.5 summarizes the factors responsible for the conversion of CPVD from no PVD and IPVD at baseline, which are age, myopia, increased axial length, and cataract surgery. Table 12.3.6 shows multiple logistic regression analyses to assess the risk factors for the conversion of CPVD. It is observed that increase in age and increased axial length were significant risk factors with OR 1.04 (1.01–1.06) and 1.34 (1.09–1.65), respectively. Subjects with myopia are at 2.13

times increased risk of developing CPVD (*P* = 0.009). Undergoing cataract surgery was associated with 2.4 times increased risk of developing CPVD (*P* = 0.031).

Table 12.3.5: Risk factors for conversion of complete posterior vitreous detachment

	Stable no PVD and IPVD (n = 243)	Conversion of CPVD (n = 140)	*P*
Age	57.58 ± 8.99	61.95 ± 9.63	< **0.001**
Gender			
Male	131 (53.9)	71 (50.7)	0.546
Female	112 (46.1)	69 (49.3)	
Duration of diabetes	8.77 ± 5.94	9.36 ± 6.93	0.402
Diabetic retinopathy			
No DR	182 (74.9)	110 (78.6)	0.416
Non STDR	43 (17.7)	22 (15.7)	0.619
STDR	18 (7.4)	8 (5.7)	0.526
DR Status Compared to baseline			
Improved	5 (2.1)	1 (0.7)	0.422
Stable	199 (81.9)	122 (87.1)	0.179
Incidence	19 (7.8)	10 (7.1)	0.810
Progressed	20 (8.2)	7 (5)	0.234
Refractive error			
Emmetropia	99 (40.7)	35 (25)	**0.002**
Myopia	57 (23.5)	53 (37.9)	**0.003**
Hyperopia	87 (35.8)	52 (37.1)	0.793
Axial length	22.78 ± 1.08	23.09 ± 1.19	**0.010**
Cataract surgery			
Not done	230 (94.7)	119 (85)	**0.001**
Done	13 (5.3)	21 (15)	

Conversion to CPVD from no PVD & IPVD at baseline

Table 12.3.6: Multiple logistic regression analysis of risk factors for the conversion of complete posterior vitreous detachment

	Stable No PVD and IPVD vs conversion to CPVD	*P*
Age	1.04 (1.01 - 1.06)	**0.002**
Gender		
Male	1	0.231
Female	1.32 (0.83 - 2.10)	
Duration of diabetes	1.00 (0.96 - 1.04)	0.708
Diabetic retinopathy		
No DR	1	
Non STDR	0.92 (0.40 - 2.09)	0.844
STDR	0.96 (0.24 - 3.78)	0.955
DR Status Compared to baseline		
Stable	1	
Incidence	0.77 (0.25 - 2.36)	0.658
Progressed	0.72 (0.19 - 2.73)	0.635
Refractive error		
Emmetropia	1	
Myopia	2.14 (1.20 - 3.81)	**0.009**
Hyperopia	1.67 (0.96 - 2.90)	0.066
Axial length	1.35 (1.10 - 1.67)	**0.004**
Cataract surgery		
Not done	1	**0.038**
Done	2.32 (1.04 - 5.16)	

Conversion to CPVD from no PVD & IPVD at baseline

12.4 Discussion

Our study shows that 80.8% of the subjects with no PVD at baseline evolved to IPVD or CPVD during the follow-up. Although IPVD tended to remain same as IPVD, 32.63% (93 subjects) progressed to CPVD. Single factor associated with conversion to IPVD was hyperopia. Risk factors for the conversion of CPVD were found to be increased with age, myopia, increase in axial length, and undergoing cataract surgery. Surprisingly, we did not find any association

between the stages of DR and the progression of DR with conversion status of PVD. This was in contrast to the earlier studies reporting that proliferation is more rapid in cases with IPVD and it promotes and aggravates the proliferation process.(41,156,157) Post hoc power analysis revealed 90.21% power in estimating the prevalence of PVD among subjects with retinopathy.

Conversion of CPVD was found to be significantly associated with increase in age, which was supported by the earlier studies that reported prevalence of PVD increases with age.(35,156,157,158) Along with liquefaction and syneresis of vitreous with age, the important pathogenesis of PVD is age-related weakening of adhesion between posterior vitreous cortex and the ILM.(159) Autopsy studies have showed that it remained attached to the retina in subjects younger than 60 years with extensive liquefaction of vitreous, suggesting that vitreous liquefaction alone is not sufficient to precipitate PVD.(160,161) Kloti et al.(162) reported that Muller cell infarction results in the dissolution of the adhesion of vitreous to the ILM. Sebag et al.(159) have postulated that progressive thickening of ILM with increase in age affects the ability of Muller cells to synthesize and maintain the components of extracellular matrix at ILM–vitreous cortex interface, thereby leading to the weakening of vitreoretinal adhesion.

We did not find any association between conversion of PVD status and gender. However, our pervious study reported a higher prevalence of PVD in men.(38) Duration of diabetes did not have any association with the conversion of IPVD and CPVD from no PVD at baseline; this result was consistent with that reported in the previous studies.(156)

Subjects undergoing cataract surgery were at 2.4 times higher risk of converting to CPVD. Earlier studies also reported the incidence of PVD following cataract surgery.(163) Even though reasons for incidence of PVD following cataract surgery are not known, various theories suggest that the anterior movement of vitreous after the lens removal might have a role in the

development of PVD.(164) Studies also reported lower concentration of hyaluronic acid in subjects with aphakia compared to those with phakic contralateral eyes, and the loss of hyaluronic acid adds to instability of vitreous and leads to development of PVD.(165)

In our study, myopia and increased axial length were found to be significant risk factors for conversion of CPVD in multiple logistic regression analyses. These results were supported by Morita et al.,(40) who reported that liquefaction of vitreous leads to development of PVD in subjects with myopia. It is also postulated that Muller cell dysfunction occurs in advanced myopic fundus degeneration and with increase in axial length, which was reported by abnormal b-wave amplitudes on electroretinography, which in turn leads to PVD.(166)

In our previous report,(38) we found an association of sight-threatening DR and PVD; however, on follow-up, we did not find any association of PVD progression and incidence with DR incidence and progression. Being a population based study, the number of subjects with DR progression and with PVD progression was less, which could be a potential limitation of the study.

To conclude, we report the progression of PVD in subjects with diabetes. Various independent risk factors for the conversion of CPVD from no PVD and IPVD are increase in age, myopia, increased axial length, and undergoing cataract surgery. We did not find any association of progression of retinopathy with PVD.

Chapter 13

13.1 Conclusion

In this population based cohort study we analyzed various structural and functional changes among subjects with diabetes with and without DR. From the structural changes based on SD-OCT, we report early neuronal changes in terms of retinal thinning and thinning of photoreceptor layer among subjects with diabetes and also conclude that superior and temporal quadrants are the most likely regions where the earliest and clinically non detectable changes in retinal thickness can be detected. We also report a trend of shallowing of foveal slope with increase in the severity of DR. Increased dark pixel values on SD-OCT scans among subjects with diabetes but no DR suggest early neuronal degeneration which occur even before the functional parameters are affected. Subjects with type II diabetes showed both generalized errors of colour discrimination and a specific pattern of blue-yellow defects. Gender adjusted prevalence of impaired colour vision among subjects with diabetes was 43%. Risk factors for impaired colour vision were females, presence of CSME, STDR and increased IOP. We developed a model to predict contrast sensitivity based on ocular and systemic factors, which explains about 30% variation in contrast sensitivity. However, longitudinal studies are required to validate the model in clinical practice. Reduced retinal sensitivity and altered fixation characteristics among subjects with diabetes but no retinopathy explains the early neuronal degenerative changes. Moderate NPDR showed a significant reduction in retinal sensitivity compared to other severe stages of DR (severe NPDR and PDR), probably due to the preponderance of retinal lesions in macular area. Various risk factors for the structural and functional changes were also reported.

13.2 Specific Contributions

To the best of our knowledge this is the first study to report various structural and functional changes among subjects with type II diabetes with and without DR in a population based cohort. We also analyzed various ocular and systemic risk factors for the changes observed in these subjects. In this study we developed a program to assess the early neuronal changes in terms of dark and light pixels and also derived a mathematical model to analyze the foveal slope parameter from SD-OCT images.

13.3 Future Scope of the Study

Assessing the structural and functional changes among subjects with diabetes even without retinopathy is important for detecting the early neuronal changes. Structural abnormalities in DR can be detected and characterized by SD-OCT and it compliments fluorescein angiography in understanding the pathology and managing it accordingly. Sequential foveal slope measurements and the dark and light pixel values assessed through SD-OCT scans may predict the progression of DR and the early diagnosis of neuronal changes even before microvascular changes appear. However further longitudinal studies are required to confirm the utility of these measurements. Impaired colour vision and contrast sensitivity among subjects with diabetes reported from our current study re-emphasizes the importance of functional vision assessment and suggest a possible need for occupational counseling. Knowing various risk factors for the structural and functional changes that occur among subjects with diabetes, there is a scope for development of new software to predict the onset of disease, so that treatment paradigms can be appropriately planned to prevent the visual disability.

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Appendix I

Medical history and biochemical investigations:

A detailed history, including data on demographics, systemic and ocular history, was obtained from all patients. Body mass index (BMI) was calculated by using the formula: weight (in kilograms)/height (in meters)^2. Waist circumference was measured with the hip taken as the greatest circumference (the widest protrusion of the hip) on both the sides and measurements were made to the nearest centimeter. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or history of use of antihypertensive medications. Resting heart rate per minute was taken after making the subject remain in a seated position for at least five minutes. The mean ocular perfusion pressure was calculated using the formula

$$\mathrm{MOPP} = \frac{2}{3}\left[\mathrm{DBP} + \frac{1}{3}\,(\mathrm{SBP} - \mathrm{DBP})\right] - \mathrm{IOP}$$

Biochemical investigations (total serum cholesterol, high-density lipoproteins, serum triglycerides, hemoglobin, HbA1c) were conducted at the base hospital with the blood samples being collected from the subjects in a state of fasting. Glycemic control was categorized as normal (HbA1c < 5.6%), good to fair (HbA1c 5.6 -8.0%) or poor (HbA1c > 8.1%). The low-density lipoprotein was calculated using the modified Friedewald formula for Indian population. Anemia was defined as a hemoglobin concentration of <13 g/dl in men and <12 g/dl in women. The subject was considered normoalbuminuric if Albumin Creatinine Ratio (ACR) was less than 30 mg/g, microalbuminuric if the ACR was between 30 and 300 mg/g, and macroalbuminuric if the ACR was above 300 mg/g respectively. Diabetic nephropathy was defined as present if the ACR is ≥30 mg/g.

Diabetic neuropathy assessment was done by measuring VPT using sensitometer (Dhansai Laboratory, India). The VPT was measured by a single observer by placing biothesiometer probe perpendicular to the distal plantar surface of the great toe of both legs. The VPT was measured at the voltage level when the subject reported the first sensation of vibration. The mean VPT measure of three readings of both legs was considered for the analysis. Diabetic neuropathy was considered as present if the VPT value was >20 V.

Comprehensive eye examination:

The following test procedures were performed for all the subjects.

Refraction

All subjects best corrected visual acuity (BCVA) was tested using modified ETDRS (Early Treatment Diabetic Retinopathy Study) chart at 4 meters and the values were converted to log MAR units for data analysis. Objective and subjective refraction were performed and the refractive error was noted in terms of spherical equivalent which is calculated as spherical error plus half of the cylindrical error.

Slit lamp biomicroscopy

Anterior segment evaluation including lids, cornea, conjunctiva and anterior chamber was done using Zeiss SL 130 (Carl Zeiss, Jena, Germany) slit lamp.

Applanation tonometry

Intraocular pressure (IOP) was recorded for all the subjects using Goldmann applanation tonometer (Zeiss AT 030 Applanation Tonometer, Carl Zeiss, Jena, Germany) in both eyes;

0.5% proparacaine eyedrops are used for topical anesthesia, and a 2% fluorescein strip to stain the tear film.

Grading of lens opacities

Grading of lens opacities was done using Lens Opacity Classification System (LOCS) III system. After the pupils were dilated with tropicamide (1%) and phenylephrine hydrochloride (2.5%) drops (instilled twice, if necessary), each subject's eyes were examined with a slit lamp. Comparing each eye with the LOCS III standard photographs (mounted close to the slit lamp), the examiner identified specific lens opacities and assigned severity grades. The lens opacities were separated into four major groups according to the photographic standards: nuclear opalescence (NO), nuclear colour (NC), cortical cataract (CC), and posterior subcapsular cataract (PSC). Intergrader agreement was determined by having both graders assess the eyes of 50 subjects recruited from the pilot study who had various grades of cataract. The grading agreements were: NO (k = 0.87), NC (k = 0.83), CC (k = 0.89), and PSC (k = 0.81). The overall average grading agreement was high (k = 0.85). A significant NC was identified by the presence of an LOCS III score of >4 for NO or >4 for NC. Similarly, a significant CC was identified by an LOCS III score of >2 for CC, and a significant PSC was identified by an LOCS III score of >2 for PSC.

Fundus examination and grading of Diabetic Retinopathy:

The binocular indirect ophthalmoscope (Keeler Instruments Inc., Pennsylvania, USA) and +20 D lens (Nikon) were used to examine the fundus after pupillary dilatation. Retinal photographs were obtained using Carl Zeiss FF 450 Plus IR Fundus Camera; all subjects underwent 45°, 4-field stereoscopic digital photography (posterior pole, nasal, superior, and inferior). For those

with any evidence of retinopathy, additional 30°, 7-field stereo digital pairs were obtained. DR was graded using Klein's classification (Modified ETDRS study scales). All photographs were graded by 2 independent observers in a masked fashion; grading agreement was high (k = 0.83).

Diabetic retinopathy grading

No DR	No abnormality
Mild NPDR	Only microaneurysm
Moderate NPDR	More than mild, but less than severe
Severe NPDR	Any of the following: 20 or more intraretinal hemorrhages in 4 quadrants, venous beading in >2 quadrants or intraretinal neovascularization in 1 quadrant
Proliferative DR	One or more of the following: neovascularization or preretinal or vitreous hemorrhage

NPDR: Non-Proliferative DR

Diabetic macular edema (DME) was defined as follows

- Absent: No retinal thickening or hard exudates in posterior pole
- Present: Some retinal thickening or hard exudates in posterior pole

Clinically significant macular edema (CSME) is defined by the ETDRS to include any of the following features:

- Thickening of the retina at or within 500 microns of the center of the macula.
- Hard exudates at or within 500 microns of the center of the macula, if associated with thickening of the adjacent retina (not residual hard exudates remaining after the disappearance of retinal thickening).
- A zone or zones of retinal thickening one disc area or larger, any part of which is within one disc diameter of the center of the macula.

Publications and Presentations

Publications from thesis:

1. **Gella L**, Raman R, Rani PK, Sharma T. Spectral domain optical coherence tomography characteristics in diabetic retinopathy. Oman J Ophthalmol. 2014;7(3):126-9.
2. **Gella L**, Raman R, Kulothungan V, Pal SS, Ganesan S, Sharma T. Impairment of Colour Vision in Diabetes with No Retinopathy: Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study (SNDREAMS- II, Report 3). PLoS One. 2015 Jun 8;10(6):e0129391.
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4. **Gella L**, Raman R, Pal SS, Ganesan S, Sharma T. Incidence, Progression, and Associated Risk Factors of Posterior Vitreous Detachment in Type 2 Diabetes Mellitus: Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study (SN-DREAMS II, Report No. 7). Semin Ophthalmol. 2015 Jul 6:1-7. [Epub ahead of print]
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7. **Gella L**, Raman R, Sharma T. Quantitative spectral domain optical coherence tomography thickness parameters in type II diabetes. Oman J Ophthalmol 2016;9:32-6.

Conference presentations related to thesis:

1. Spectral Domain Optical Coherence Tomography and Microperimetric Values in Normal Indian Eyes. March 2009, Dr E. Vaithilingam Memorial Scientific Session, Chennai.
2. Structural changes in diabetic retinopathy using spectral domain optical coherence tomography. August 2010, Elite International Vision and Optometry Conference, Chennai.
3. Characterization of Scotoma in Patients with Clinically Significant Macular Edema. January 2011, Asia ARVO, Singapore.
4. Prevalence of posterior vitreous detachment in population with type II diabetes mellitus and its influence on diabetic retinopathy. March 2012, Dr E. Vaithilingam Memorial Scientific Session, Chennai.
5. Contrast Sensitivity and its Determinants among Subjects with Diabetes: SN-DREAMS II. August 2015, Elite International Vision and Optometry Conference, Chennai.

Other Publications

1. Nittala MG, **Laxmi G**, Raman R et al. Spectral-Domain OCT and Microperimeter Characterization of Morphological and Functional Changes in X-linked Retinoschisis. Ophthalmic Surg Lasers Imaging 2009; 40: 71-74.
2. Verma A, Rani PK, Raman R, Pal SS, **Laxmi G** et al. Is Neuronal dysfunction an early sign of diabetic retinopathy? Microperimetry and spectral domain optical coherence

tomography (SD-OCT) study in individuals with diabetes, but no diabetic retinopathy. Eye 2009; 1-8

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15. Nagesha CK, Raman R, **Gella L**, Sharma T. Optic Pit Maculopathy: A Review of Literature and Suggested Treatment Algorithm. Kerala state Ophthalmic Journal. 2014 Sept; vol 26, No 3:211-8.
16. Jaichandran VV, Raman R, **Gella L**, Sharma T. Local Anesthetic Agents for Vitreoretinal Surgery: No Advantage to Mixing Solutions. Ophthalmology. 2015 May;122(5):1030-3.
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18. Raman R, Nittala MG, **Gella L**, Pal SS, Sharma T. Retinal sensitivity over hard exudates in diabetic retinopathy. J Ophthalmic Vis Res 2015;10:160-4.

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20. Raman R, Pal SS, Ganesan S, **Gella L**, Vaitheeswaran K, Sharma T. The prevalence and risk factors for age-related macular degeneration in rural-urban India, Sankara Nethralaya Rural-Urban Age-related Macular degeneration study, Report No. 1. Eye (Lond). 2016 Feb 26. Epub ahead of print.

21. Raman R, **Gella L**, Srinivasan S, Sharma T. Diabetic retinopathy: An epidemic at home and around the world. Indian J Ophthalmol. 2016;64:69-75.

Brief Biography of the Candidate

Ms. Gella Laxmi graduated from Bausch and Lomb School of Optometry, Hyderabad in 2007. She did her M.Phil. in Optometry from Elite School of Optometry, Chennai in 2009. She has published around 28 research articles in peer reviewed indexed journals and has presented in around 10 national and international conferences. She received Elite School of Optometry Alumni Association (ESOAA) award for the best research publication of the year 2011-2012. She received award for best research presentation at All India Optometry Conference (AIOC) at Amritsar, in 2008. She also received a travel grant for the poster presentations at Indian Eye Research Group (IERG) 2008 at Madhurai. She is an optometrist cum research scholar practicing at Diabetic retinopathy project, Vision Research Foundation since 2007. She is also part of various epidemiological projects conducted at diabetic retinopathy department. She is actively involved in teaching B.S Optometry students and guiding projects. She also holds the post of reviewer for few peer reviewed journals. Her area of interests includes clinical research, diabetic retinopathy, diagnostics and imaging.

She has currently enrolled for PhD program on “Structural and Functional Correlations in Diabetic Retinopathy” with Birla Institute of Technology and Science, Pilani.

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He has made extensive scientific contributions in ophthalmology with > 100 peer reviewed publications, about 10 book chapters. He has contributed a chapter in the renowned book, Retina, 5th Edition. Stephen Ryan. He has been a guide for DNB, MPhil and PhD for over a decade and has guided >25 thesis. He is a reviewer in many national and international peer reviewed journals. He is a member of Govt. of India National Taskforce for Diabetic Retinopathy and one of the five National experts in the Certificate course of evidence based management of Diabetic Retinopathy conducted by Public health foundation of India. He is an Associate-Editor of Indian Journal of Ophthalmology and a member of Editorial board of Community Eye health (South Asia edition). He has received research grants from World Diabetes Foundation (WDF), RD TATA trust, LCIF, Google and Anglia Ruskin University for his research work on Diabetic retinopathy.

Brief Biography of the Co-supervisor

Dr. Kunda MM Rao joined Indian Institute of Science, Bangalore in 1973. He was responsible for the design and development of Black and White and Colour Drum Scanner Imager, first time in India. He worked with National Remote Sensing Center (NRSC) in various positions, as Operations Director IRS-1C in 1996 and IRS-1D in 1997, Deputy Director (Data Processing Area) at NRSC, till December 2006. As an Adjunct Faculty, BITS-Pilani, Hyderabad campus taught Image Processing and Remote Sensing courses till 2015.

His areas of interest include Satellite Data Processing, Image Processing, Medical Imaging, Data Mining and Photo Writing. He has designed and developed number of technologies for Image Processing and Remote Sensing applications. He obtained five patents for his designs. He has designed and developed software to analyze planimetric parameters of optic disc to study glaucoma disease objectively. He designed software to montage fundus images to study retinal diseases during 1996. He has developed multimodal image registration and fusion techniques for satellite images and for medical images. He has got several awards for his inventions

He is Fellow of Institute of Electronics & Telecommunications (IETE) India, Fellow of ISNT, Life Senior Member IEEE (US), Life Member Indian Society of Remote Sensing (ISRS). He has more than 130 research publications/reports to his credit. He has supervised over 200 graduate and undergraduate students in their research projects. Three of his students got PhD from JNTU Hyderabad. He is currently supervising number of PhD and M.S students. He has edited and brought out a Special Issue of International Journal of Applied Earth Observation and Geoinformation on Indian Remote Sensing Satellite, Resourcesat-1. www.drkmm.com

STRUCTURAL AND FUNCTIONAL CORRELATIONS IN DIABETIC RETINOPATHY

THESIS

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Chapter 13

13.1 Conclusion

In this population based cohort study we analyzed various structural and functional changes among subjects with diabetes with and without DR. From the structural changes based on SD-OCT, we report early neuronal changes in terms of retinal thinning and thinning of photoreceptor layer among subjects with diabetes and also conclude that superior and temporal quadrants are the most likely regions where the earliest and clinically non detectable changes in retinal thickness can be detected. We also report a trend of shallowing of foveal slope with increase in the severity of DR. Increased dark pixel values on SD-OCT scans among subjects with diabetes but no DR suggest early neuronal degeneration which occur even before the functional parameters are affected. Subjects with type II diabetes showed both generalized errors of colour discrimination and a specific pattern of blue-yellow defects. Gender adjusted prevalence of impaired colour vision among subjects with diabetes was 43%. Risk factors for impaired colour vision were females, presence of CSME, STDR and increased IOP. We developed a model to predict contrast sensitivity based on ocular and systemic factors, which explains about 30% variation in contrast sensitivity. However, longitudinal studies are required to validate the model in clinical practice. Reduced retinal sensitivity and altered fixation characteristics among subjects with diabetes but no retinopathy explains the early neuronal degenerative changes. Moderate NPDR showed a significant reduction in retinal sensitivity compared to other severe stages of DR (severe NPDR and PDR), probably due to the preponderance of retinal lesions in macular area. Various risk factors for the structural and functional changes were also reported.

13.2 Specific Contributions

To the best of our knowledge this is the first study to report various structural and functional changes among subjects with type II diabetes with and without DR in a population based cohort. We also analyzed various ocular and systemic risk factors for the changes observed in these subjects. In this study we developed a program to assess the early neuronal changes in terms of dark and light pixels and also derived a mathematical model to analyze the foveal slope parameter from SD-OCT images.

13.3 Future Scope of the Study

Assessing the structural and functional changes among subjects with diabetes even without retinopathy is important for detecting the early neuronal changes. Structural abnormalities in DR can be detected and characterized by SD-OCT and it compliments fluorescein angiography in understanding the pathology and managing it accordingly. Sequential foveal slope measurements and the dark and light pixel values assessed through SD-OCT scans may predict the progression of DR and the early diagnosis of neuronal changes even before microvascular changes appear. However further longitudinal studies are required to confirm the utility of these measurements. Impaired colour vision and contrast sensitivity among subjects with diabetes reported from our current study re-emphasizes the importance of functional vision assessment and suggest a possible need for occupational counseling. Knowing various risk factors for the structural and functional changes that occur among subjects with diabetes, there is a scope for development of new software to predict the onset of disease, so that treatment paradigms can be appropriately planned to prevent the visual disability.