COLOUR VISION IN DIABETES

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DECLARATION

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ABSTRACT

Diabetes Mellitus (DM) has become one of the most important metabolic diseases that reduces one's quality of life and doubles the risk of early death. Amongst the major complications linked to DM, diabetic retinopathy (DR) leads to gradual loss of vision and blindness. DR is now the second cause of certifiable blindness among the working age adults in the UK. The lifetime costs to the UK government are calculated to be up to £327,000 per person, with almost 50% of these costs being attributed to loss of productivity caused by visual impairment and blindness. The UK is one of the leading countries in the implementation of DR screening programmes. The latter rely heavily on fundus imaging and grading using trained experts and subsequent referral to hospital for further clinical examination and evaluation depending on the grade of retinopathy. It is now known that subtle, structural changes in the retina that are linked to diabetes can precede detectable vascular changes. The former can affect one's colour vision and this offers the potential of using changes in chromatic sensitivity as an early biomarker of retinal disease.

The first part of this thesis focuses on measuring chromatic sensitivity using the colour assessment and diagnosis (CAD) test in diabetic subjects with varying degrees of retinopathy. The severity of colour vision loss is graded in comparison to other factors that are normally linked to diabetes, such as the type, grade, control methods and duration. The results of this study reveal losses of both red / green and yellow / blue chromatic sensitivity in patients with diabetes, but the correlation with factors, normally associated with high risk of diabetes is low. The results from this study do, however, show that the magnitude of chromatic sensitivity losses correlates with the severity of diabetic retinopathy.

The second, related study examines the effectiveness of intravitreal injection of a dexamethasone implant (Ozurdex) in patients with diabetic macular oedema (DMO) in stabilising and reducing loss of visual function and in particular the reduction in chromatic sensitivity up to 24 weeks. This treatment demonstrated efficacy in the treatment of chronic DMO and DMO which is resistant to anti-VEGF treatment. The results show that intravitreal treatment with Ozurdex causes improvement is visual acuity, central retinal thickness and significant improvement in red / green chromatic sensitivity.

ABBREVIATIONS

µm	Micro metres
ACCORD	Action to Control Cardiovascular Risk in Diabetes Study
BSCVA	Best Spectacle Corrected Visual Acuity
CAD	Colour Assessment and Diagnosis Test
CARDS	Collaborative Atorvastatin Diabetes Study
CST	Central Subfield Thickness
CIE	Commission Internationale d'Eclairage
CS	Contrast Sensitivity
CV	Colour Vision
CRT	Cathode Ray Tube
CSMO	Clinically Significant Macular Oedema
DCCT	Diabetes Control and Complications Trial
DD	Disc Diameter
dLGN	Dorsal Lateral Geniculate Nucleus
DM	Diabetes Mellitus
DMO	Diabetic Macular Odema
DN	Diabetic Neuropathy
DR	Diabetic Retinopathy
DRIVE UK	Diabetic Retinopathy in Various Ethnic groups in UK
DRS	Diabetic Retinopathy Study
DSS	Diabetic Screening Service
EDIC	Epidemiology of Diabetes Interventions and Complications study
ERG	Electroretinography
ETDRS	Early Treatment Diabetic Retinopathy Study
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes Trial
HbA1C	Glycosylated Haemoglobin, Type A1C
IDF	International Diabetes Federation
IOP	Intraocular pressure
IPL	Inner Plexiform Layer
IR	Infra-Red
IRAS	Integrated Research Application System
IRMA	Intraretinal microvascular abnormality
IS	Inner Segment
ISPED	Italian Society of Paediatric Endocrinology and Diabetology
L cone	Long wavelength sensitive cone
LOCS III	Lens Opacities Classification System III
M cone	Medium wavelength sensitive cone
mmol	Milli moles per Litre
MO	Macular Oedema
MPOD	Macular Pigment Optical Density

MPOD	Macular Pigment Optical Density
MSVI	Moderate and Severe Visual Impairment
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
Nm	Nano metres
NPDR	Non-proliferative Diabetic Retinopathy
NSC	National Screening Committee
NVD	New vessels on disc
NVE	New vessels elsewhere
OCT	Optical Coherence Tomography
OD	Optical Density
OPL	Outer Plexiform Layer
OS	Outer Segment
PDR	Proliferative Diabetic Retinopathy
PRP	Pan-retinal Laser Photocoagulation
RG	Red-Green
RGC	Retinal Ganglion Cell
RPE	Retinal Pigment Epithelium
RT	Retinal Thickness
S cone	Short wavelength sensitive cone
SNU	Standard Normal Units
STED	Sight Threatening Eye Disease
SVL	Severe Visual Loss
TNF-α	Tumour Necrosis Factor-α
UK	United Kingdom
UKADS	United Kingdom Asian Diabetes Study
UKPDS	United Kingdom Prospective Diabetes Study
UV	Ultra-Violet
VEGF	Vascular Endothelial Growth Factor
WESDR	Wisconsin Epidemiology Study of Diabetic Retinopathy
WHO	World Health Organization
YB	Yellow-Blue
VA	Visual Acuity

1 THE VISUAL SYSTEM

1.1 ANATOMY OF THE EYE

The eye is located within the orbital cavity surrounded by muscles, nerves, vessels and fatty tissue. It is made up of segments of two spheres of different sizes, the anterior, smaller segment is transparent and forms one-sixth of the eyeball while the posterior, larger segment is opaque and forms about five-sixths of the eyeball.

The eyeball consists of three layers (Figure1-1):

1. The fibrous layer, composed of the sclera and cornea
2. The vascular pigmented layer, composed of the uvea (iris, ciliary body and choroid)
3. The nervous layer, i.e. the retina

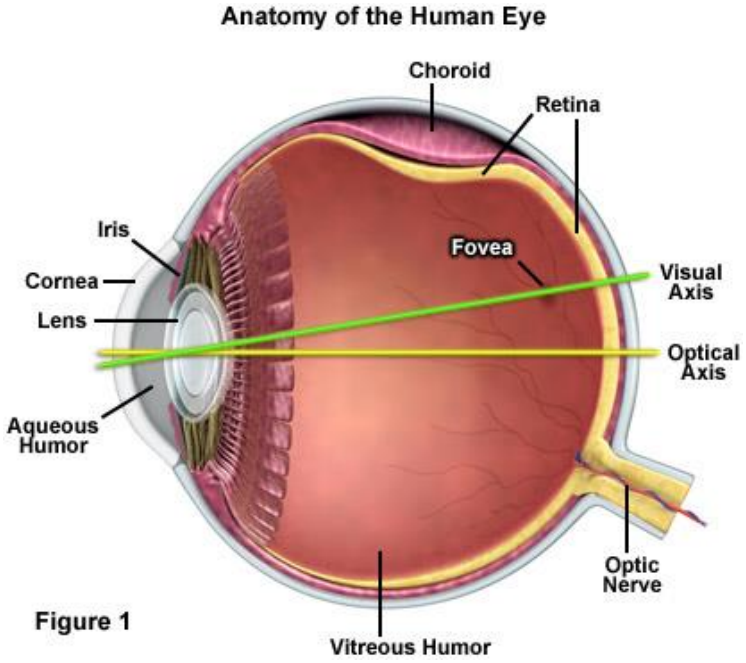


Figure 1-1: Anatomy of the human eye (http://zeiss-campus.magnet.fsu.edu)

1.1.1 The fibrous layer

The sclera forms the posterior five-sixths of the eyeball and is opaque; it is about 1mm thick posteriorly, thinning at the equator to 0.6mm (Snell and Lemp, 2001). The sclera may be divided into three layers, the episclera, the scleral stroma and the lamina fusca. The main function of the sclera is to provide a tough external framework that protects the intraocular contents from trauma and together with the intraocular pressure it helps preserve the shape of the eyeball. The sclera joins the cornea at the corneoscleral junction.

The cornea is transparent; it forms the anterior one-sixth of the eyeball. The average corneal diameter is 10.6 mm vertically and about 11.7 mm horizontally. It is the main structure responsible for the refraction of light entering the eye. The average corneal thickness is 530 microns (Doughty and Zaman, 2000). It consists of 5 layers: the epithelium, Bowman's layer (membrane), the stroma, Descemet's membrane and the endothelium. The transparency of the cornea results from its avascularity and the uniform spacing of the collagen fibrils in the stroma. It transmits most of the visible and infrared light (IR) (300-2500 nm) with the maximum transmittance between 500-1300 nm.

1.1.2 The vascular pigmented layer

The uvea is the middle vascular layer of the eyeball. It consists of the iris, the ciliary body and the choroid. The iris is a thin pigmented diaphragm with a central aperture called the pupil. The pupil varies in diameter from 1 to 8 mm. The iris divides the space between the lens and the cornea into an anterior and

a posterior chamber. The colour of the iris varies from light blue to dark brown depending on the amount of pigment in the melanocytes. The iris controls the amount of light entering through the pupil through its contractile ability to dilate and constrict the pupil. During accommodation for near vision, the pupil constricts to restrict the incoming light to the central part of the lens thus diminishing the spherical aberration. The periphery of the iris is attached to the anterior surface of the ciliary body.

The ciliary body is a triangular structure; it is made up of the ciliary epithelium, the ciliary stroma and the ciliary muscle. The anterior surface of the ciliary body gives rise to the ciliary processes that produces the aqueous humour. The zonular fibres of the lens pass from the lens equator to attach to the anterior surface of the ciliary body in the intervals between the ciliary processes. Contraction of the ciliary muscle moves the apex of the ciliary processes towards the lens equator and relaxes the lens zonules, thus allowing accommodation (Figure 1-2).

The choroid lies posterior to the ciliary body. It extends from the optic nerve posteriorly to the ciliary body anteriorly. It is extremely vascular and divided into three layers: the vessel layer, the capillary layer and Bruch's membrane. The main function of the choroid is to provide the outer retina and the retinal pigment epithelium (RPE) with oxygen and nutrients through its choriocapillaries.

The crystalline lens is a transparent biconvex structure situated behind the iris and pupil in front of the vitreous body. The lens is enveloped within an elastic capsule formed of type IV collagen fibrils embedded in a matrix of glycoproteins and sulphated glycosaminoglycan and suspended by zonules. The transparency of the lens is attributed to its avascularity, permeability of its capsule and its

pump mechanism. It accounts for 30% of the eye's refractive power. It transmits light from the ultra-violet (UV) (300 nm) part of the spectrum to the 1900 nm range in the IR part.

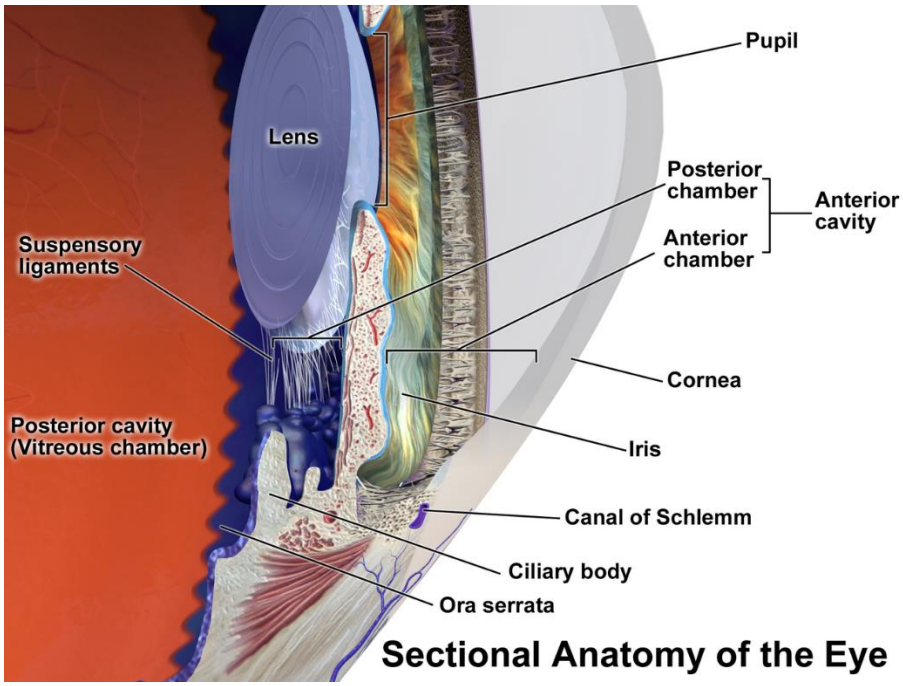


Figure 1-2: Sectional anatomy of the eye (Blausen gallery, 2014).

1.1.3 The nervous layer – The retina

The retina is the internal layer of the eyeball. It is where the neural processing of the optical image is initiated. It is continuous with the optic nerve posteriorly and it extends anteriorly to become the epithelium of the ciliary body and the iris. It consists of an outer pigmented segment and an inner neurosensory segment, these two segments are formed of 10 layers (Figure 1-3).

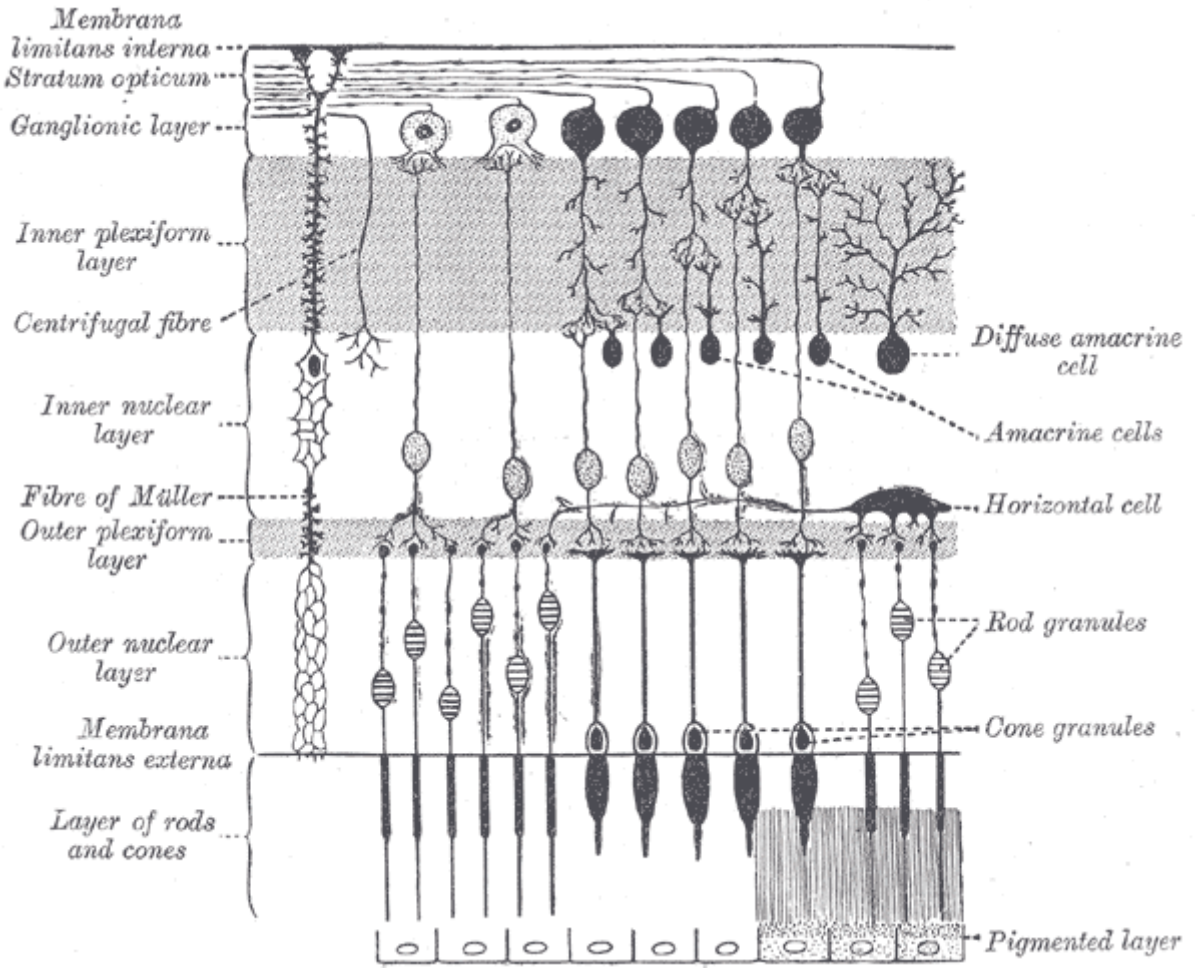


Figure 1-3: The different layers of human retina (adapted from Gray's anatomy, 1918)

The RPE is the outermost layer of the retina, and has several functions including the phagocytosis of the outer segments of the photoreceptors, the participation in the formation of rhodopsin and its dark pigmentation absorbs the light thus reducing the amount of light scatter in the eye.

The main structures in the retina are the optic disc, the macula lutea and the peripheral retina (ora serrata). The optic disc is the point at which the optic nerve fibres carrying the visual signals exit the eye by piercing the sclera.

The macula lutea is an oval yellowish area at the centre of the retina. This yellowish colour is attributed to the yellow xanthophyll carotenoids, zeaxanthin and lutein. It measures about 5 mm in diameter and its centre lies about 5.5 mm temporal to the optic disc. The central depressed area in the centre of the macula is called the fovea; it is about 1.5 mm in diameter. The foveola is the central part of the fovea and has a diameter of 0.35 mm. The sharpest vision occurs at the macula because of its avascularity and dense cone mosaic.

The neurosensory segment of the retina is formed of three main groups of neurons: the photoreceptors, the bipolar cells and the ganglion cells.

1.2 PHOTORECEPTORS

There are two types of photoreceptors, the rods and the cones. The rods are mainly responsible for vision in dim light (scotopic conditions), while the cones are adapted to bright lights (photobic conditions) and can resolve fine details and colour vision (CV). There is three different types of cones named short-, middle- or long-wavelength, or S, M and L cones. This classification is based on the sensitivity of the photopigments to short, middle or long wavelength light.

There are about 110 to 125 million rods in the retina and about 6.3 to 6.8 million cones. The rods are absent at the foveola rising rapidly in number towards the periphery with the opposite happening with the cones which are most dense at the fovea and then decrease in number towards the periphery.

Rods and cones have four distinct structural regions: outer segment (OS), inner segment (IS), cell body and synaptic terminal (Figure 1-4). The OS is filled with about 600 to 1000 membrane discs that contain the photochemical rhodopsin

molecules in the rods and photopsin molecules in the cones. The OS is connected to the IS by a connecting stalk that contains modified cilium. The IS in both rods and cones consists of two areas, the ellipsoid, which is situated next to the connecting stalk and the myoid located toward the vitreous, they contain numerous mitochondria, ribosomes and Golgi apparatus that provide the necessary energy for photo-transduction. The cell body of the photoreceptors is connected through microtubules to pear-shaped spherule in rods and expanded end in cones - called cone pedicles that contain many presynaptic vesicles and synapse with the dendrites of the bipolar cells.

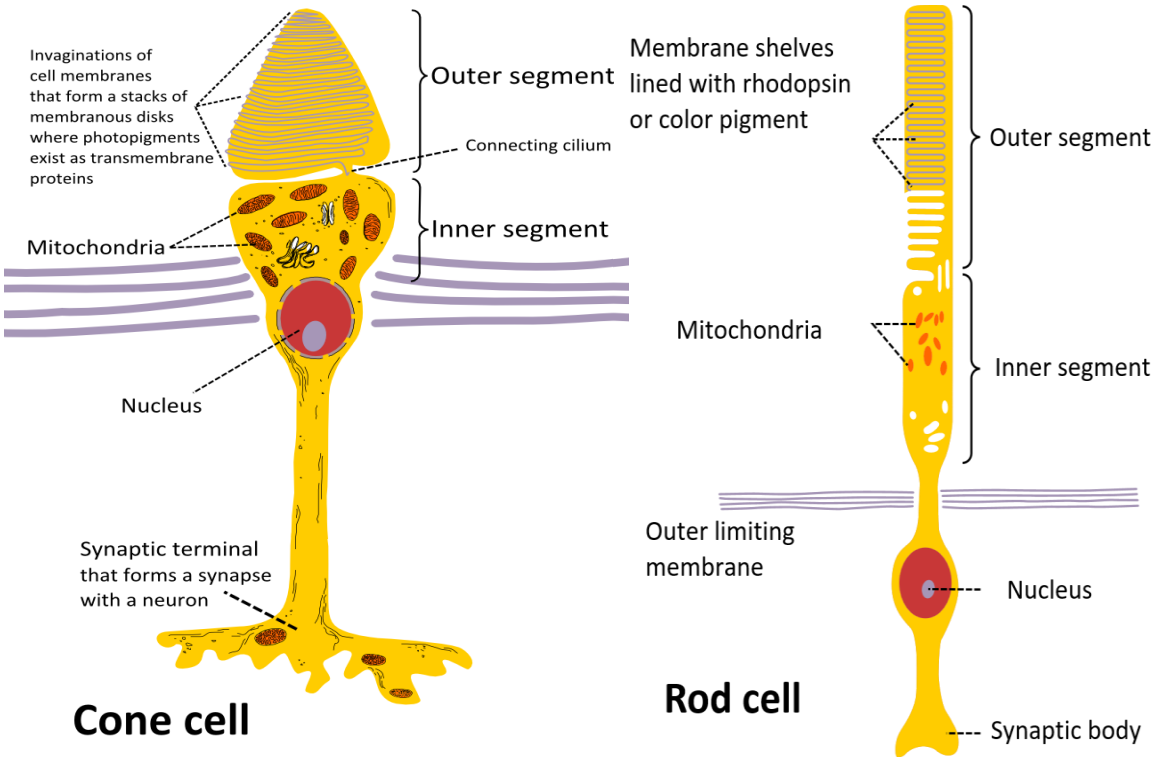


Figure 1-4: The main structure of rods and cones; the photopigment is located in the outer segment (OS). The OS is connected to the IS by a connecting stalk that contains modified cilium. At the base of the cell the spherule contain the presynaptic vesicles that synapse with the dendrites of the bipolar cells (created by Ivo Kruusamägi, https://upload.wikimedia.org/wikipedia/commons/4/48/Cone_cell_eng.png).

When light falls on the retina, photon absorption by visual pigment rhodopsin triggers isomerization of 11 *cis* retinal to *all trans* retinal resulting in activation of rhodopsin that starts chain of actions with closure of the ion channels and inducing change in the electric potential of the photoreceptor. This will continue until *trans* is transformed back to *cis*. Similar biochemical events occur in cones. The signals generated are transmitted through the synapses.

1.3 INTRA-RETINAL PATHWAYS

Two main patterns exist for transmitting signals from photoreceptors, the vertical pathway and the lateral pathway. In the vertical pathway, rods and cones synapse with bipolar and ganglion cells. First post receptoral synapses takes place between the photoreceptors and the bipolar cell, which in turn synapses with a ganglion cell. The first synapse at the outer plexiform layer (OPL) is responsible for processing static information. The second synapse at inner plexiform layer (IPL) is responsible for processing phasic information. Signals from 120 rods converge to one ganglion cell while signals from 6 cones converge to one ganglion cell, this becomes one-one ratio at the fovea. The lateral pathway for transmitting the signal involves the horizontal cells and amacrine cells which allows signals from neighbouring photoreceptors to transmit the signals through other bipolar or ganglion cells (Bye et al., 2013).

There are separate channels for detecting the luminance and processing colour, they are called ON and OFF channels. In response to light, all photoreceptors hyperpolarize, hyperpolarization can either pass unchanged in the OFF bipolar cells or it can either be depolarized in the ON bipolar cells. The ganglion cells have a circular receptive field that allows convergence of the signals from

different areas of the retina. The receptive field have a centre surround organization, ON-centre ganglion cells are excited at the centre and inhibited in the periphery, while OFF-centre ganglion cells are inhibited in the centre and excited in the periphery. Rods are only connected to depolarizing bipolar cells (ON).

The neural circuit output through S-cone have two pathways, either ‘branch 1’: straight through to small bistratified ganglion cell pathway or ‘branch 2’: through horizontal cells to the terminals of adjacent L and M cones which then output to ganglion cells via L and M specific bipolar cells (Figure 1-5). The presence of L or M cones in the center of the receptive field, together with input from either the ON or OFF ganglion cell will result the four possible combinations of hue perception involving contribution from the three different types of cones in the following manner: blue = (S+M)-L; yellow = L-(S+M); red = (S+L)-M and green = M-(S+L).

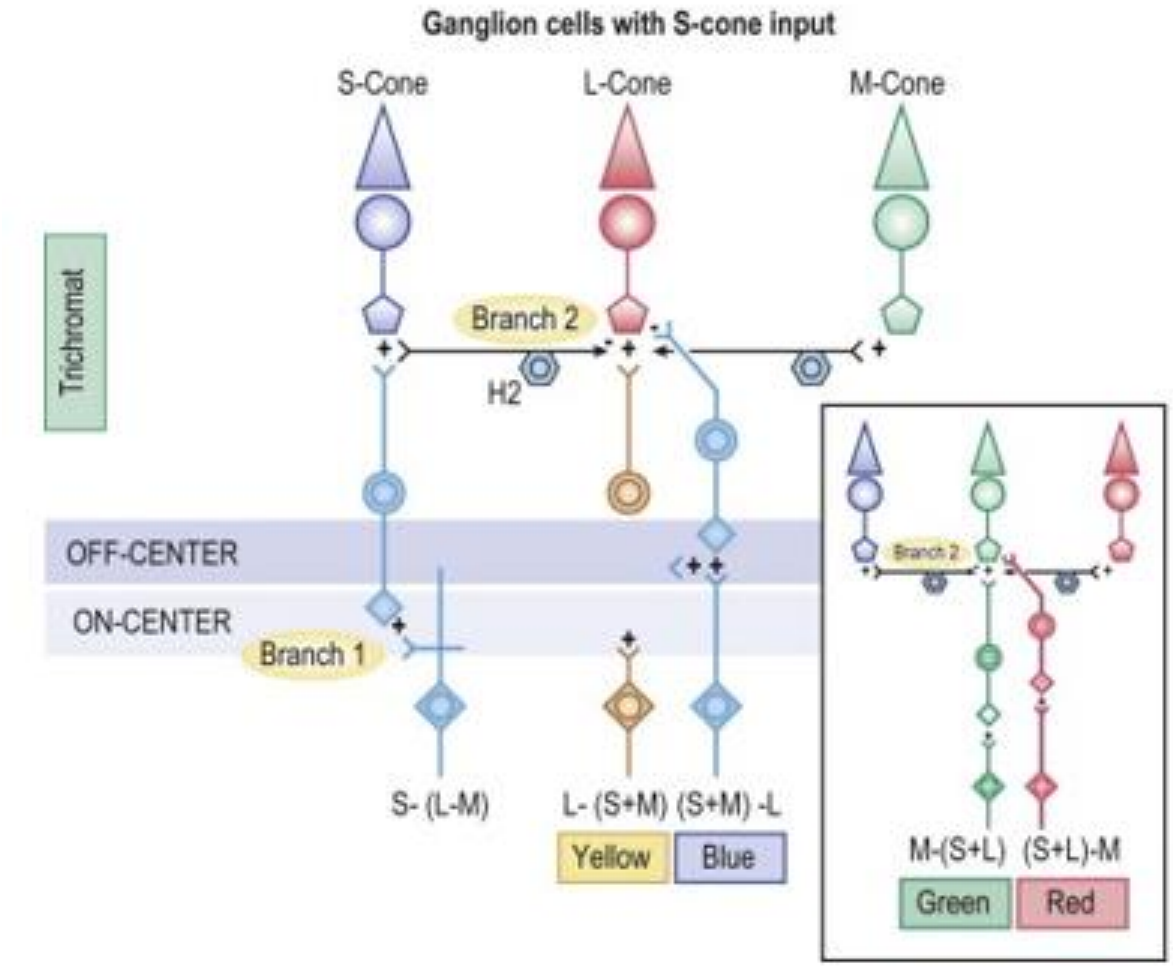


Figure 1-5: The Retinal circuits based on known anatomical connections. These circuits would give rise to signals matching the spectral signatures of human perception following addition of a third cone type. 'Branch 1' indicates the straight-through S-cone output to the small bistratified ganglion cell pathway ; 'branch 2' is a second known anatomical connection that may provide S-cone output to the midget ganglion cell pathway via H2 horizontal cells. The addition of M cones changes the 'surrounds' of small bistratified ganglion cell receptive fields. Both the 'center' and 'surround' of ganglion cell receptive fields become altered by the addition of M cones, splitting the pre-existing blue-yellow circuit into two organizations with distinct spectral response properties, one for blue-yellow and one for red-green (inset). (Neitz, Jay, Adler's Physiology of the Eye, Chapter 34 'colour vision', 648-654).

1.4 POST-RETINAL PATHWAYS

Nerve fibres converge at the optic disc to leave the eye through the optic nerve to reach the optic chiasm, where the nerve fibres from the nasal retina decussate to enter the optic tract of the opposite side; whereas the temporal fibres remain uncrossed. The fibres continue through the optic tracts which carry the signals to the dorsal lateral geniculate nucleus in the brain (dLGN).

The dLGN has a laminated structure and consists of six curved layers of cells. The layers of cells are separated by white bands of optic nerve fibres; they are numbered 1 to 6. The nerve fibres that cross the midline terminate in layers 1, 4 and 6, while the fibres that do not cross terminate in layers 2, 3 and 5.

The optic radiations are formed of nerve fibres that originate from the nerve cells in the laminae of the LGN. They terminate in the visual cortex (V1) which lies in the posterior part of the occipital cortex and is formed of two areas, the primary visual area (Brodmann's area 17) and the secondary visual area (Brodmann'e areas 18 and 19).

1.5 VISUAL PROCESSING OF NORMAL TRICHROMATIC COLOUR VISION

Colour vision is one of the most important aspects of visual function. An individual with three classes of normally functioning cone photoreceptors is usually described as having normal, trichromatic colour vision. This relates to a process called 'trichromacy' in which the retina processes and conveys colour information starting by the light stimulation of the 3 different types of cones.

The trichromatic colour theory was first suggested by Thomas Young in the 18th century, and later developed by Hermann von Helmholtz. This theory states that normal colour signals require the presence of three kinds of nerve fibers in the retina.

Later in 1875, Hering suggested that large number of colours is perceived by mixture of two opponent colours, this was called the opponent colour theory (Figure 1-6). It involves the formation of colour through comparison of signals from different cones through two channels. The RG channel that utilise the difference between L and M cones. The YB channel which involves comparing signals derived from the M and L cones to the ones received through S cones. The combination of three cones photopigments with their different photosensitivity enables the individual to process a great number of combinations allowing the appreciation of large number of different colours. (Figure 1-6).

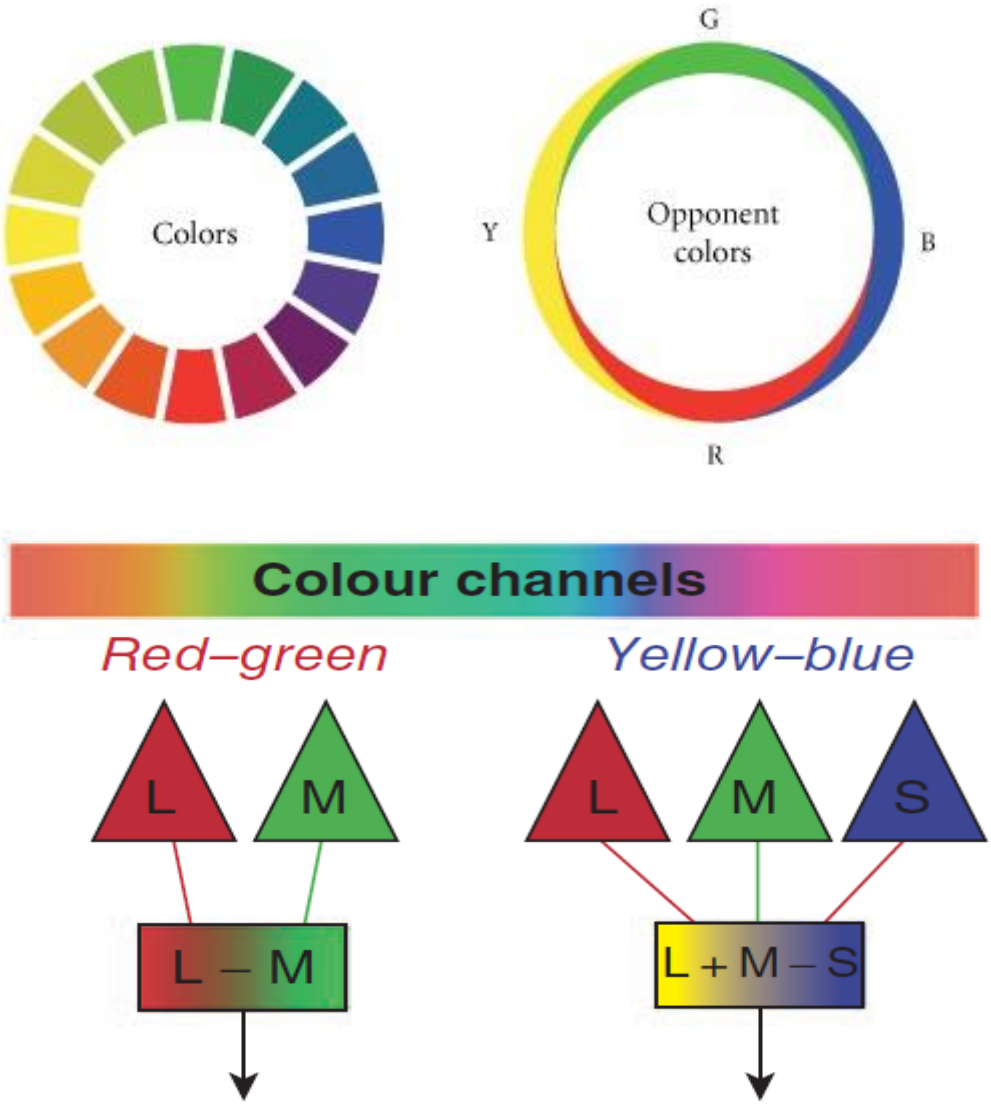


Figure 1-6: Hering's opponent colours schematic diagram. A diagrammatic representation of opponent colours theory. The right ring shows a range of colours changing in small steps from green at the top clockwise to blue, red, yellow and back to green. The left ring shows the hypothetical contributions of each of the colour-opponent pairs (red vs. green, and blue vs. yellow) to the appearance of the corresponding colours in the right ring. Comparison from the M and L cone signals produce the RG channel while the YB channel is achieved from comparison of signals from S cone to the combined M and L signals. (Hering, 1920).

Utilising this theory and the trichromatic colour theory, in 1896 Muller proposed the zone theory or stage theory which is based on three different colour receptors that initiates three different neuronal signals that are processed and transformed as two sets of opponent colour signals.

The zone theory assumes that there is three zones, in the first zone there is three types of cones initiating colour vision through absorption of light by photopigments sending electrical signals to reach the second zone. In the second zone colour signals are coded creating one achromatic signal and two achromatic signals following the opponent theory proposed by Hering. The final zone is located in the cortex and is responsible for interpretation of the signals in relation to other spatial and temporal information. (Figure 1-7)

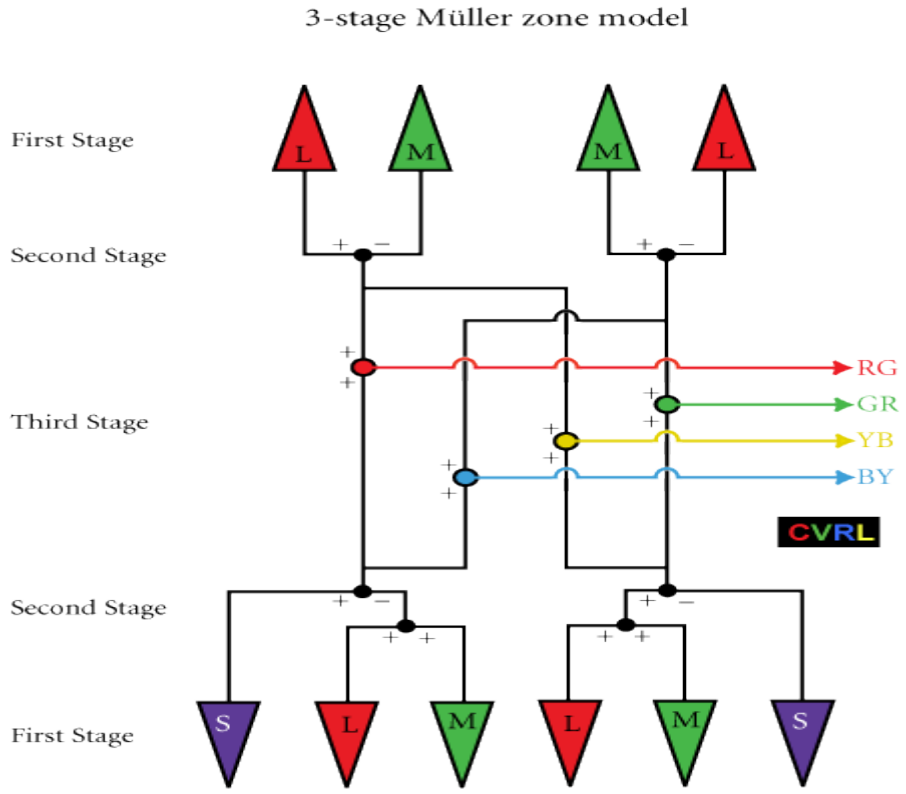


Figure 1-7: Schematic representation of Müller zone model. *First stage:* L, M and S cone photoreceptors (top and bottom). *Second stage:* L-M and M-L cone opponency (top) and S-(L+M) and (L+M)-S cone opponency (bottom). *Third stage:* colour is achieved by summing the various cone-opponent second stage outputs (obtained from hhtp://www.cvrl.org).

In 1956 Gunnar Svaetichin proved the existence of the retinal nerve cells that Young suggested, these cells are sensitive to three different wavelengths. Later in 1980 Bowmaker & Dartnall were able to use micro-spectroscopy to measure the spectral transmittance of a small beam of light passing through the outer segment (OS) of individual cones *in vitro* and obtain three kinds of spectra with their absorbance peaks. Each of the three types of cones contains a different type of photosensitive pigment, which is composed of a transmembrane protein called opsin and a light-sensitive molecule called 11-cis retinal. Colour processing starts when each of these three different photosensitive pigments is struck by a photon with the specific wavelength to which that pigment is most sensitive to, this initiates a cellular response and a signal that is transmitted to the brain.

The spectral sensitivities of the three cone classes (Figure 1-8) along with the spectral sensitivity of the rods, their wavelengths are as follows: rods, 496.3+/- 2.3 nm; red cones, 564 +/- 5.2 nm; green cones, 534 +/- 3.5 nm; and blue cones 420 +/- 3.6 nm (Stockman and Sharpe, 2000).

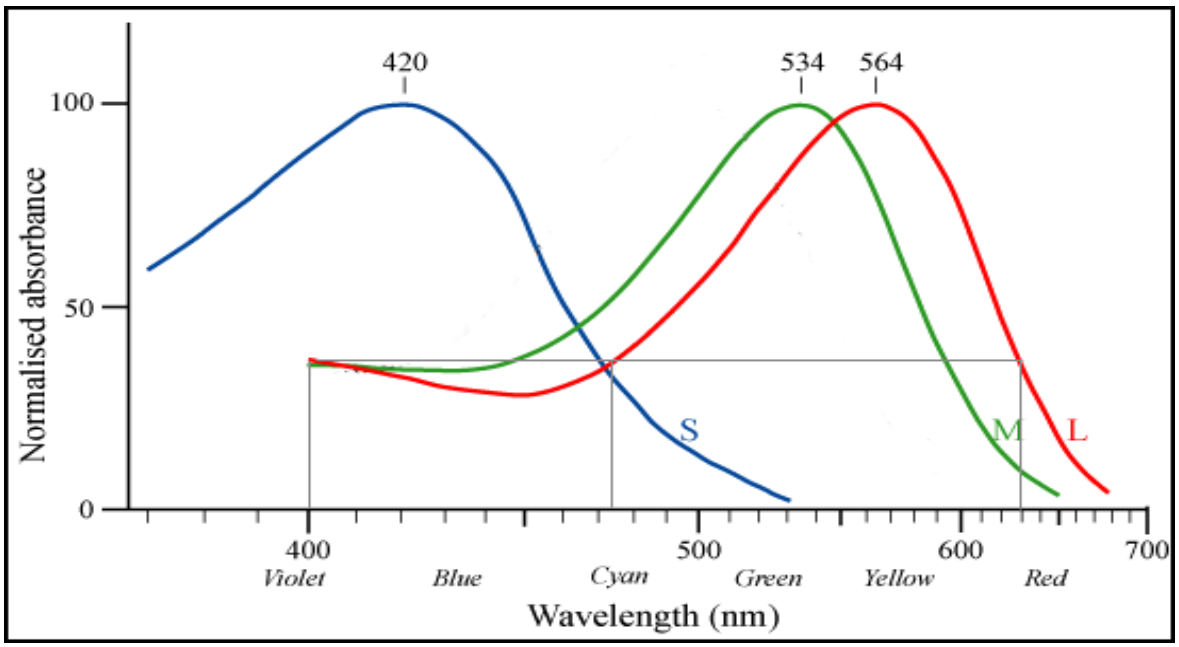


Figure 1-8: Normalized absorbance of the three human cone pigments, indicating the relative sensitivity of the three cone types to light of different wavelengths (obtained from http://www.cvrl.org).

More recently Berson et al., 2002 described another subtype of retinal ganglion cell that contains melanopsin and is photosensitive. It is believed that such intrinsically photosensitive cells contribute to the control of the circadian rhythm and its maximum spectral absorption is 484 nm.

2 INTRODUCTION

2.1 DIABETES MELLITUS

Diabetes Mellitus (DM) is one of the most serious conditions of the modern age, its incidence with the subsequent complications are increasing worldwide. According to the international diabetes federation (IDF), 415 million are affected with diabetes; this number is expected to rise to 642 million people by 2040. DM is a chronic metabolic disease that results from defects in insulin secretion, insulin action or both. The world health organization (WHO) issued recommendations for the diagnostic criteria of DM based on clinical symptoms such as polyuria, polydipsia and unexplained weight loss in addition to blood tests that show:

1. Random venous plasma glucose concentration ≥ 11.1 mmol/l or
2. Fasting plasma glucose concentration ≥ 7.0 mmol/l or
3. Plasma glucose concentration ≥ 11.1 mmol/l, two hours after 75g anhydrous glucose in an oral glucose tolerance test (OGTT)

There are different types of DM, the main types as identified are:

1. Type I:

   An autoimmune condition in which the body attacks and destroys Beta islet cells of Langerhans in the pancreas which are insulin producing cells. This type affects 10% of people with diabetes.

2. Type 2:

   The body does not produce sufficient insulin or there is insulin resistance which means that different body cells are not able to respond adequately to normal levels of insulin. This type affects 90% of people with diabetes.

3. Gestational diabetes:

   It develops in woman during pregnancy where the fasting glucose level is ≥5.6 mmol/l or 2-hour plasma glucose level ≥7.8 mmol/l.

Complications of diabetes:

Hyperglycaemia as well as hypoglycaemia have damaging effect on different cells of the body systems leading to macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (Diabetic nephropathy, neuropathy, and retinopathy).

2.2 DIABETIC RETINOPATHY

2.2.1 DEFINITION OF DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is the most common and serious ocular complication of diabetes; it is a chronic progressive potentially sight threatening disorder caused by changes in the retinal microvasculature which are brought about by prolonged hyper and hypoglycaemia. DR is accelerated by other diabetes mellitus related factors such as duration, control and associated systemic conditions such as Hypertension and Dyslipidaemia (Van Leiden et al., 2002).

2.2.2 CLASSIFICATION OF DIABETIC RETINOPATHY

DR is diagnosed and graded according to the extent of microvascular changes observed on fundus examination. This is now done through diabetic retinal screening programmes in different parts of the country through the National Health Service (NHS). The United Kingdom (UK) has a well implemented screening programme that offers screening to 80.1% of its diabetic population with 73.5% actually undertaking the screening tests (NHS Diabetic Eye Screening Programme, 2012). All eligible patients have their fundus photographs taken, this is later reviewed by a grader who is able to quantify the severity of changes and refer to a specialist when needed.

There are two widely used forms for DR grading in the UK, one of which has been adopted from the National Screening Committee (NSC) (Harding et al., 2003) and used in population screening programmes, and another which is based on the Early Treatment Diabetic Retinopathy Study (ETDRS) that depend on modifications from the Diabetic Retinopathy Study (DRS) and mainly used in clinical settings. There are similarities between both forms of classifications with focus on two aspects, retinopathy and maculopathy.

2.2.2.1 Retinopathy

Retinopathy is classified according to the vascular changes present in the retina. Table 2-1, summarises the classification criteria according to the NSC grading criteria and table 2-2 summarises classification criteria according to the ETDRS.

2.2.2.2 Maculopathy

Maculopathy is a form of retinopathy that affects the macula and is also known as diabetic Macular Oedema (DMO). It is classified according to the NSC into presence (M1) or absence (M0) of maculopathy. The ETDRS define clinically significant Macular Oedema (CSMO) by the presence of the following:

- Retinal thickening within 500 µm of the center of the fovea
- Hard, yellow exudates within 500 µm of the center of the fovea with adjacent retinal thickening
- At least 1 disc area of retinal thickening, any part of which is within 1 disc diameter of the center of the fovea

R0	**None**	
R1	**Background**	Microaneurysm(s)
		Retinal haemorrhage(s) ± any exudate
R2	**Pre-proliferative**	Venous beading
		Venous loop or reduplication
		Intraretinal microvascular abnormality (IRMA)
		Multiple deep, round or blot haemorrhages
		Cotton wool spots (CWS)
R3	**Proliferative**	New vessels on disc (NVD)
		New vessels elsewhere (NVE)
		Pre-retinal or vitreous haemorrhage
		Pre-retinal fibrosis ± tractional retinal detachment
M0	**Nil present**	
M1	**Maculopathy**	Exudate within 1 disc diameter (DD) of the centre of the fovea
		Circinate or group of exudates within the macula
		Retinal thickening within 1DD of the centre of the fovea (if stereo available)
		Any microaneurysm or haemorrhage within 1DD of the Centre of the fovea only if associated with a best visual acuity of ≤ 6/12 (if no stereo)
PHOTOCOAGULATION (P)		Focal/grid to macula
		Peripheral scatter
UNCLASSIFIABLE (U)		Ungradable / unobtainable

Table 2-1: National Screening Committee grading criteria (Harding et al., 2003).

Disease Severity Level	Findings Observable upon Dilated Ophthalmoscopy
Mild non-proliferative retinopathy	At least one microaneurysm, and definition not met for moderate nonproliferative retinopathy, severe nonproliferative retinopathy, early proliferative retinopathy, or high-risk proliferative retinopathy (see below)
Moderate non-proliferative retinopathy	Haemorrhages and/or microaneurysms ≥ standard photograph 2A; and/or soft exudates, venous beading, or intraretinal microvascular abnormalities definitely present; and definition not met for severe nonproliferative retinopathy, early proliferative retinopathy, or high-risk proliferative retinopathy (see below)
Severe non-proliferative retinopathy	Soft exudates, venous beading, and intraretinal microvascular abnormalities all definitely present in at least two of fields four through seven; or two of the preceding three lesions present in at least two of fields four through seven and haemorrhages and microaneurysms present in these four fields, equalling or exceeding standard photo 2A in at least one of them; or intraretinal microvascular abnormalities present in each of fields four through seven and equalling or exceeding standard photograph 8A in at least two of them; and definition not met for early proliferative retinopathy or high-risk proliferative retinopathy (see below)
Early proliferative retinopathy (i.e., proliferative retinopathy without Diabetic Retinopathy Study high-risk characteristics)	New vessels; and definition not met for high-risk proliferative retinopathy (see below)
High-risk proliferative retinopathy (proliferative retinopathy with Diabetic Retinopathy Study high-risk characteristics)	New vessels on or within one disc diameter of the optic disc (NVD) ≥ standard photograph 10A* (about one-quarter to one-third disc area), with or without vitreous or preretinal haemorrhage; or vitreous and/or preretinal haemorrhage accompanied by new vessels, either NVD < standard photograph 10A or new vessels elsewhere (NVE) ≥ one-quarter disc area

Table 2-2: Classification of diabetic retinopathy in the early treatment of diabetic retinopathy study (ETDRS report 7, 1991).

2.2.3 DIABETIC MACULAR OEDEMA (DMO)

DMO is one of the most serious squeals of DR. It affects 20% of patients with DR (Yau et al., 2012) and is caused by a breakdown of the outer blood retinal barriers secondary to an inflammatory process that releases prostaglandins, leukotrienes and vascular endothelial growth factor (VEGF) (Bhagat et al., 2009). This causes an accumulation of extracellular fluid within the inner and outer retinal layers, creating cystoid spaces that can extend to affect the whole retinal thickness. This leads to displacement of the retinal neuronal components with subsequent temporary or permanent visual loss (Pelosini et al., 2011). Implications and management of DMO are discussed in more details later in the thesis in chapter 5.

2.2.4 EPIDEMILOLOGY OF DIABETES AND DIABETIC RETINOPATHY

2.2.4.1 Incidence and prevalence of diabetes

The number of people suffering from diabetes is on the rise and it is estimated that 220 million people have diabetes worldwide (WHO, 2010). According to a recent DR guidelines report published by the Royal College of Ophthalmologists it is estimated that 1 in every 25 people suffer from diabetes in the UK (https://www.rcophth.ac.uk).

2.2.4.2 Incidence & prevalence of diabetic retinopathy

It is estimated that worldwide there are more than 100 million people with DR and of these at least 30 million people are at risk of developing severe sight-threatening retinopathy (Yau at al., 2012). In 2004, Resnikoff et al. reported that DR is the leading cause of visual impairment amongst the working age population in the developed world. In the UK, it is one of the three most common recorded causes of certification as sight impaired (Bunce et al., 2010). In a recent study about prevalence and causes of vision loss in high-income countries and in Eastern and Central Europe: 1990-2010 (Bourne et al., 2014), DR was the fifth most common cause of moderate and severe vision impairment (MSVI) (presenting visual acuity <6/18 but ≥3/60 in the better eye) and blindness (presenting visual acuity <3/60).

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), a large population study on DR conducted in the USA found that the overall 10-year incidence of retinopathy was 74%. 64% of people with retinopathy at baseline showed progression of retinopathy on follow ups and 17% progressed to develop proliferative retinopathy (Varma, 2008). After 25 years follow up, in the type 1 diabetes cohort, almost all patients (97%) developed retinopathy over time with 43% developing proliferative retinopathy and 29% developing macular oedema (MO).

In the last 15 years of the study there was decline in the yearly incidence and progression of DR rate compared to the first 10 years of the study, especially in people with recently diagnosed diabetes (Klein et al., 2009). This demonstrates the positive impact of improved diabetes management in developed countries

during the past two decades. Since the introduction of the nationwide DR screening programmes in England and Wales and for the first time in at least the last five decades, DR is now the second cause of certifiable blindness among the working age adults (Liew at al., 2013).

In the Liverpool Diabetic Eye Study, Younis et al., 2002 studied the prevalence of diabetic eye disease in patients entering a systematic primary care-based eye screening programme. They recruited 831 subjects with type 1 diabetes and 7231 subjects with type 2 diabetes. In the type I group, 45.7% had non-proliferative diabetic retinopathy (NPDR), 3.7% had proliferative diabetic retinopathy (PDR) and 16.4% had sight threatening eye disease (STED). For the type 2 group, 25.3% had NPDR, 0.5% had PDR and 6.0% had STED.

The United Kingdom Prospective Diabetes Study (UKPDS) ran for twenty years (1977-1997) and recruited 5,102 patients with newly diagnosed type 2 diabetes. As part of this study, Kohner et al., 1998 reported the incidence of retinopathy, defined as microaneurysms or worse lesions in at least 1 eye, in 39% of men and 35% of women, while marked retinopathy with cotton wool spots or intraretinal microvascular abnormalities was present in 8% of men and 4% of women from a total of 2964 of Caucasian patients included in the study. The UKPDS also showed that intensive blood-glucose control with sulphonylurea or insulin therapy reduced the risk of any diabetes-related endpoint by 12% and microvascular disease by 25%. The post-trial monitoring showed continuing benefit of earlier improved glucose control with reduction in diabetes related endpoint (9%, P=0.04) and microvascular disease (24%, P=0.001) as well as continuing benefit of earlier metformin therapy, with maintenance of the relative risk reductions reported for any diabetes related endpoint (21%, P=0.013).

2.2.5 PATHOPHYSIOLOGY OF DIABETIC RETINOPATHY

DR is caused by a chronic exposure to hyperglycaemia and other risk factors such as hypertension which seems to initiate a cascade of biochemical and physiological changes that are responsible for the retinal microvascular damage (Figure 2-1).

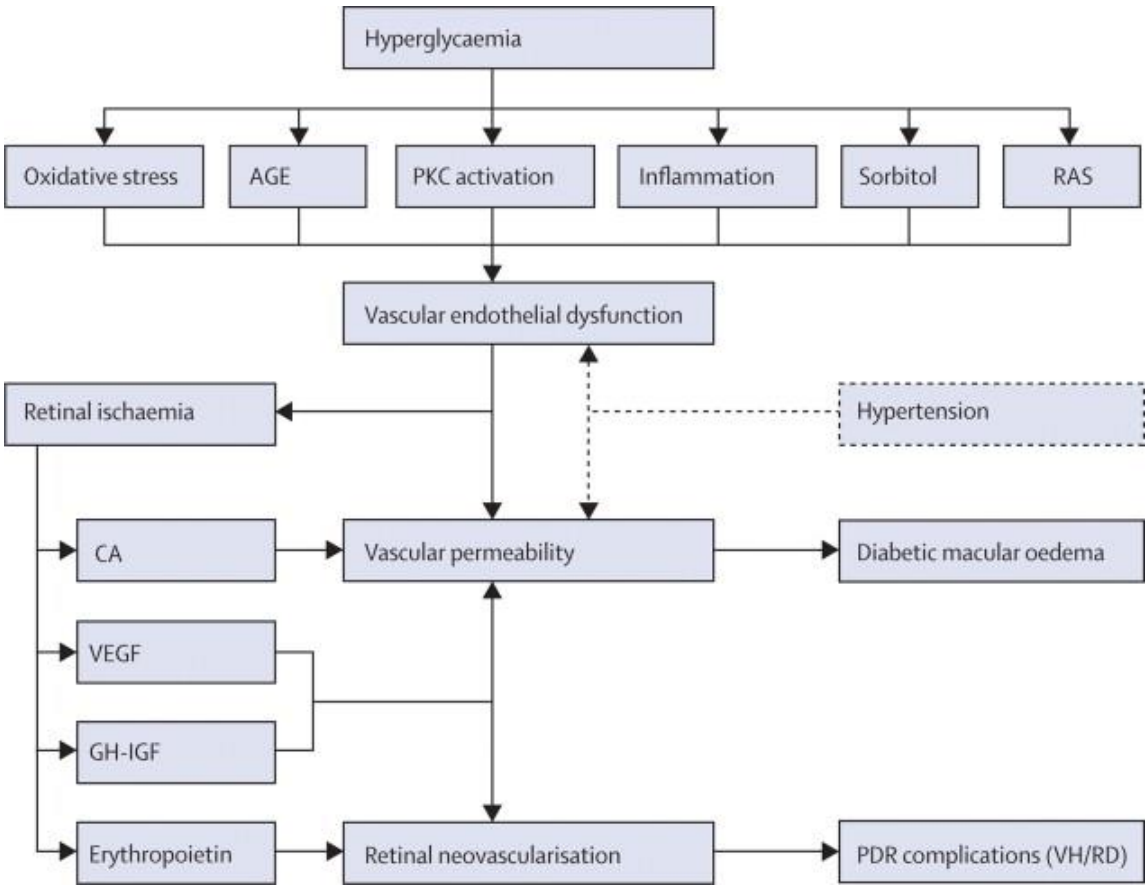


Figure 2-1: Diagrammatic representation of pathophysiology of diabetic retinopathy. Hyperglycaemia instigates a cascade of events leading to retinal vascular endothelial dysfunction. Resultant retinal ischaemia and increased vascular permeability, augmented by hypertension, are two key common pathways underlying development of vision-threatening diabetic retinopathy. AGE=advanced glycation end-products. PKC=protein kinase C. RAS=renin-angiotensin system. CA=carbonic anhydrase. VEGF=vascular endothelial growth factor. GH-IGF=growth factor–insulin growth factor. PDR=proliferative diabetic retinopathy. VH=vitreous haemorrhage. RD=retinal detachment (Cheung et al., 2010).

As response to the hyperglycaemic state, inflammatory mediators are upregulated triggering responses that cause abnormal leucocyte-endothelial interactions resulting in microvascular damage. The structural changes observed in retinal vasculature starts with arteriolar dilation in response to the increased capillary pressure this result in leakage (oedema and hard exudates) and rupture (haemorrhages).

2.2.5.1 Vascular changes vs. neuronal changes

The understanding of the mechanisms involved in developing DR have been increasing over the past few decades. It is now accepted that DR is not just the result of microvascular changes but it also involves neuronal retinal changes (Antonetti et al, 2006). There have been many studies that used electroretinography (Wachtmeister at al., 1978) (Holopigian et al., 1997) (Fortune et al., 1999), contrast sensitivity (Della Sala et. al., 1985) (Di Leo et al., 1992) (safi et al., 2017) (Nerianuri et al., 2017) , retinal senstivity (Montesano et al., 2017) (Nerianuri et al., 2017) and CV tests to demonstrate that neuroretinal function is compromised before the onset of vascular changes are observed.

The retina is vascularized neural tissue, not just a network of blood vessels. It remains unclear whether the sequence of events starts in blood vessels or neural cells. Inflammation is a prominent component of DR and is a natural response to continuous cellular stress. It is characterized by increased vascular permeability, oedema, cytokine and chemokine expression including vascular endothelial growth factor (VEGF), interleukin-1β and tumour necrosis factor-α (TNF-α). Although these normal inflammatory factors serve as an adaptive

mechanism to maintain neuronal function and represent an attempt to limit the retinal damage over time they cause tissue destruction and neovascularization with retinal damage (Seigel et al., 2000; Barber at al., 2001; Gariano et al., 2005).

2.2.5.2 Risk factors

Risk factors for developing DR can be divided to two subgroups, non-modifiable and modifiable factors which are described below.

2.2.5.2.1 Non-modifiable risk factors

1. Ethnic origin:

The Diabetic Retinopathy in Various Ethnic groups in UK (DRIVE UK) study (Sivaprasad et al., 2012) was the largest cross-sectional study on the prevalence of DR in the various ethnic groups with diabetes in the UK. It showed that the prevalence of any retinopathy in type 2 diabetes is highest in people of African/Afro-Caribbean descent compared to South Asians or white Europeans. In type 2 diabetes, the prevalence of any DR was 38.0% in white Europeans compared to 52.4% in African/Afro-Caribbeans and 42.3% in South Asians. Similarly, sight threatening DR was also significantly more prevalent in Afro-Caribbeans (11.5%) and South Asians (10.3%) compared to white Europeans (5.5%).

A community-based cross-sectional study (sub-study of the UK Asian Diabetes Study (UKADS)) looked at people of South Asian ethnicity in Coventry-England.

It recruited 1035 patients with type 2 diabetes: 421 of South Asian and 614 of white European ethnicity. Results showed that people with a south Asian ethnic origin were more likely to have DR than white European people. DR was detected in 414 (40%) patients (189 South Asian (45%) versus 225 white European (37%); *P* = 0.0078). Sight-threatening retinopathy was detected in 142 (14%) patients (68 South Asian (16%) versus 74 white European (12%); *P* = 0.0597) (Raymond et al., 2009).

2. Duration and age at onset:

The duration of diabetes is a strong predictor for the development and progression of retinopathy (Fong et al., 2004). The development of DR has a linear relationship with the duration of diabetes, so the longer the duration of diabetes the higher the prevalence of DR (Wong et al., 2009).

The Wisconsin Epidemiology Study of Diabetic Retinopathy (WESDR) showed that the 4-year incidence of developing proliferative retinopathy in the younger-onset group increased from 0% during the first 5 years to 27.9% during years 13–14 of diabetes and after 15 years, the incidence of developing PDR remained stable. It also showed that in patients diagnosed before age 30 years, 97% had retinopathy and 25% had PDR at 15 years post diagnosis (Klein et al., 1984).

3. Puberty:

When the onset of diabetes is at puberty, the risk of developing DR is higher, taking into consideration that there are several physiological and psychosocial factors that can disrupt glycaemic control. The Diabetes Study Group of the Italian Society of Pediatric Endocrinology and Diabetology (ISPED) found that the prevalence of DR was higher in pubertal than in prepubertal patients, both for any grade DR (71% vs. 40%, *P* = 0.002) and for mild or more severe DR (*P* = 0.005).

Diabetes duration after menarche is associated with 30% excess risk of retinopathy compared with diabetes duration before menarche (WESDR) (Klein et al., 1990). It has also been suggested that prepubertal years might offer some protection against developing DR (Olsen et al., 2004).

2.2.5.2.2 Modifiable risk factors

1. Diabetic control:

The Diabetes Control and Complications Trial (DCCT, 1982-93) compared the effects of two treatment regimes, standard therapy and intensive control on the complications of diabetes, it demonstrated that achieving glycaemic levels close to the non-diabetic range by intensive treatment reduced the risk of developing DR by 76%. This was followed by the Epidemiology of Diabetes Interventions and Complications study (EDIC, 1994-2006) which investigated the long-lasting effects of the previously assigned therapies. One of the most important conclusions of the study was that the salutary effect of intensive therapy persists for at least 10 years after differences in glycaemia between the original intensive and conventional therapy groups have disappeared, this have been named imprinting or metabolic memory.

The UK Prospective Diabetes Study (UKPDS, 1998) showed that intensive blood glucose control reduced the risk of diabetic complications, the greatest effect being on microvascular complications by 25%. It also demonstrated that for every percentage point decrease in Glycosylated Haemoglobin, Type A1C (HbA1C) there was a 35% reduction in the risk of microvascular complications.

2. Blood pressure:

The UKPDS also studied the influence of tight blood pressure control with an ACE inhibitor or a beta blocker over a median of 8.4 years. It showed reduced risk of both microvascular and macrovascular disease, where 34% had reduction in progression of retinopathy and 47% reduced risk of deterioration in visual acuity of three lines after 10 mmHg systolic and 5 mmHg diastolic reduction in blood pressure.

Gallego et al., 2008 provided evidence for the relationship between elevated blood pressure and risk of retinopathy in young people with type 1 diabetes. They examined 1869 patients with type 1 diabetes and a median age of 13.4 years. Retinopathy developed in 36% of participants. The risk was higher for developing DR in patients who had higher systolic and diastolic blood pressures.

3. Dyslipidaemia:

Dyslipidaemia has also been linked to the pathogenesis of DR (van Leiden et al., 2002; Klein et al., 2002). Several studies have studied the effect of lipid lowering medications on the pathogenesis of DR. Sen at al. (2002) conducted a double-blind randomized placebo-controlled trial and concluded that simvastatin significantly retards the progression of retinopathy in diabetic patients although there was limited to no improvement of visual acuity in patients receiving the treatment.

The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, a randomised controlled trial with 9795 participants aged 50-75 years, found that Fenofibrate, a lipid modifying agent, reduced the need for laser treatment (5·2% *vs* 3·6%, p=0·0003) (Keech et al., 2005).

These findings were also supported by the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study which showed that over a 4-year period there was

a 40% reduction in the odds of progression of retinopathy in a subgroup having Fenofibrate in combination with a statin, compared to simvastatin alone (Chew et al., 2010).

However, the Collaborative Atorvastatin Diabetes Study (CARDS) did not find atorvastatin to be effective in reducing DR progression (Thomason et al., 2004; Colhoun et al., 2004).

2.2.6 MANAGEMENT AND TREATMENT OF DIABETIC RETINOPATHY

A systematic review and met-analysis by Cheung at al., 2009 looked at the rates of progression in DR during different time periods. They included 28 studies published between 1975 and February 2008. They found that participants in 1986–2008 studies had lower proportions of PDR and non-PDR at all-time points than participants in the 1975–1985 studies. In studies reporting outcomes at 10 years, rates for PDR were 11.5% in 1975–1985 versus 6.6% in 1986–2008 and for severe vision loss (SVL), corresponding rates were 6.0% versus 2.6%, respectively.

This data is supportive of the premise that in recent years there has been decline in the rates of DR development due to the improved screening and management of diabetes.

2.2.6.1 Management of diabetic retinopathy

The aim of the screening process and management of DR is the prevention, detection and treatment of sight-threatening maculopathy and proliferative retinopathy.

Systematic population screening for DR has been implemented in the UK several years ago (NHS diabetic eye screening programme, 2013). Screening is conducted using high-definition fundus photography which is evaluated by graders that applies strict grading criteria, as discussed previously in section 2.2. Table 2-3 summarizes the NSC management criteria.

Those patients with referable retinopathy, i.e. maculopathy, moderate, severe or PDR are usually referred within set time limits to the hospital eye service for further management as required. Not all patients referred to hospital will require treatment, regular monitoring and adequate control of diabetes and other risk factors such as blood pressure and lipid levels, can sometimes be enough to control DR changes and reduce the risk of progression to diabetic maculopathy. This requires a multi-disciplinary team approach with liaison between the DR services, ophthalmologists, diabetologists and general practitioners.

The mainstay for treating proliferative DR remains to be the pan-retinal laser photocoagulation (PRP). In some cases of advanced PDR, with vitreous haemorrhage and tractional retinal detachment, vitrectomy should be considered. As for DMO, several treatment modalities are currently available- as discussed in section 5.1 - focal or grid macular laser, still have a role in treating localized MO, although for diffuse MO intravitreal injections such as VEGFs

inhibitors or steroids are more effective and are now available and licensed by the National Institute for Health and Care Excellence (NICE) for treating DMO.

RETINOPATHY (R)	
R0	Annual screening
R1	Annual screening, inform diabetes care team
R2	Refer to hospital eye service
R3	Fast-track referral to hospital eye service
MACULOPATHY (M)	
M0	Annual screening
M1	Refer to hospital eye service
PHOTOCOAGULATION (P)	
P1	New screen — refer to hospital eye service
	Quiescent post treatment — annual screening
OTHER LESIONS (OL)	Refer to hospital eye service or inform primary physician
UNGRADABLE (U)	Media opacity hospital eye service
UNSCREENABLE/UNOBTAINABLE	
	Poor view but gradable on biomicroscopy, refer hospital eye service
	Unscreenable, discharge, inform GP
	(Option to recall for further photos if purely technical failure)

Table 2-3: National Screening Committee management criteria / action (Harding et al., 2003).

2.3 DIABETIC RETINOPATHY AND COLOUR VISION

In recent years, our understanding of DR has changed and it is now accepted that DR not only affects the retinal vasculature, but it also affects the neural retina. Hence, more studies have started investigating the neurodegenerative changes of DR. Those changes are usually assessed mainly by measuring any reduction in chromatic sensitivity or / and by using electrophysiological diagnostic tests such as full-field or multifocal electroretinography (ERG).

2.3.1 Retinal neurodegeneration in diabetes

Several changes are observed in neuronal retina in diabetes, some of those changes might even precede the development of signs that can be seen on examining the retina in DR. The mechanisms by which such changes occur are not fully understood, and although several theories exist, none have succeeded in giving a full explanation of the exact mechanisms involved. The following section lists and discusses the most common theories put forward to account for the neurodegenerative changes and events that might happen in the retina of a diabetic person.

2.3.1.1 Inflammation:

Inflammation is one of the key processes involved in DR (Racquel et al., 2005). Under normal conditions microglia which are associated with retinal neurones are regulated, but under the stress caused by diabetes, the microglia are

activated and start to release inflammatory cytokines (Schroder et al., 1991). This leads to the start of an inflammatory process that leads to the development of DR. The events that follow cause the blood–retinal barrier to degrade and in turn this may affect the normal functioning of the retina. These events include: increased vascular permeability, cellular infiltration, release of cytokines and growth factors including IGF-1, Interleukin-1, TNF and VEGF. These factors provide neurotrophic functions and are released in an attempt to repair the retinal cells and to help overcome the stress (Seigel et al., 2000; Barber at al., 2001; Gariano et al., 2005). The abundant release of these factors results in loss of the integrity of the blood-retinal barrier, progressive vascular damage and neuronal cell loss seen in progressive stages of DR (Antonetti et al., 2006).

2.3.1.2 Apoptosis

Several studies suggested that diabetes causes chronic loss of inner retinal neurones by increasing the frequency of apoptosis. This was demonstrated by Barber et al. (2003) in a study which examined animal models and post-mortem human tissue and found that the number of apoptotic nuclei in retinal neurons and glial cells was elevated after inducing diabetes. After a period of intensive treatment with insulin, the amount of apoptosis was however reduced. There are also reports from biochemical studies which show that the expression of Bax (BCL-2 associate X protein), a protein associated with degenerative diseases and caspases, an enzyme involved in apoptosis are both increased in the retinas of diabetic rats (Podesta et al., 2000; Mohr et al., 2002).

Another factor that plays a role as well in the process of apoptosis is the decrease in the anabolic stimulus provided by insulin due to the reduction in number of insulin receptors (Yi et al., 2005). It is known that insulin plays an important role in supporting retinal neurones and vascular cells (Barber et al., 2001; Kondo et al., 2003). The DCCT study demonstrated that intensive treatment with insulin reduces the amount of apoptosis and reduces the risk of developing retinopathy (DCCT, 1982-93). These findings suggest that the increase in apoptosis within the neural retina is an important factor in the onset and progression of the DR process.

2.3.1.3 Glutamate Excitotoxicity

Neuronal cells plays an important role in converting neurotoxin glutamate into less toxic glutamine which explains why excessive levels of glutamate are usually seen in the central nervous system when there is neuronal cell loss (Gillies et al., 2000). In the retina, diabetes reduces the ability of the retina to convert neurotoxic glutamate into less toxic glutamine due to the reduction of the glial enzyme glutamine synthetase (Lieth et al., 1998). There is also an associated reduction in the activity of glutamate transporters in Muller cells leading to elevated concentration of glutamate in the extra cellular fluid (Li and Puro, 2002). This observation also explains the detection of increased levels of glutamate in the vitreous in diabetic subjects (Ambati et al., 1997; Lieth et al., 1998). Neuronal apoptosis observed in the retina in patients with diabetes may therefore be caused by chronic exposure to the glutamate excitotoxicity (Barber et al., 2001).

Over the years there have been many studies to investigate the relationship between CV loss and diabetes, but the majority of them are using CV tests that were primarily designed to detect hereditary CV loss rather than pathological loss. In addition, none of the tests has the ability to quantify reliably the severity of red / green (RG) and yellow / blue (YB) colour vision loss. This is important when the aim is to follow up patients with diabetes and to detect progression or even improvement as we will discuss later in this thesis (chapter 6).

The agreement among the different studies is that there is a degree of CV loss in diabetic patients, but that the wide variation in results makes it difficult to establish how CV loss correlates with other parameters that are used routinely for screening and examining diabetic patients such as VA and OCT. Also there is no agreement as to whether these CV losses are correlated to different diabetes related factors such as grade of retinopathy, duration or control.

In 1982 Maloney and Drury studied 66 patients (132 eyes) with type I diabetes for CV loss using the FM-100 hue test and reported high error scores in 88 eyes (56.7%). Several other studies followed (Green et al., 1985; Bresnick et al., 1985; Roy et al., 1986; Trick et al., 1988; Tregear et al., 1994). In these studies, the FM 100-Hue error scores showed no correlation with age, sex, age of onset, duration of diabetes, metabolic control or with the degree of retinopathy.

However not all studies have reported similar findings. For example, Roy et al., (1984) studied 12 type 1 diabetics and found a correlation between the FM-100 error score and the degree of retinopathy, but not with fasting blood sugar. Ismail & Whitaker (1998), reported that the mean error score was increased in subjects with diabetes when compared with normal subjects, and that the level

of the mean error score increased with the severity of DR. These results have since been replicated by other studies such as Fong et al., (1999) and Barton et al., (2004) which studied patterns of hue discrimination impairment among 2701 of the diabetic patients enrolled in the ETDRS. Fong et al., reported that approximately 50% of the patients had CV scores worse than 95% of the normal population. They found that hue discrimination was associated with MO severity, age and neovascularization. In the Barton et al. study, they were able to detect 13 patterns of impaired hue discrimination and reported losses in YB axis that correlated with the severity of DR.

In a more recent study, Feitosa-Santana et al., (2006) investigated CV in 32 subjects with type II diabetes without DR and 20 age matched controls using the Farnsworth D-15 and Lanthony D-15d tests and employed triadic procedure that enabled detection of very mild CV impairment. Results were constructed in two-dimensional RG and YB perceptual opponent systems. The authors found that the colour space configurations were compressed along the YB as well as RG axes in the diabetic subjects which they attributed to the reduced photoreceptor sensitivity.

Another recent study used a computerized CV test (Arden colour contrast test) (Al Saeidi et al., 2013) to evaluate its efficacy in detection of DMO. The authors examined 42 diabetic patients with and without MO, they found that YB loss was more pronounced than RG loss and correlated with retinal thickness on the Optical Coherence Tomography (OCT).

O'Neill-Biba et al., (2010) used the CAD test on 7 diabetic subjects with no or mild DR to demonstrate that measurement of reduced chromatic sensitivity

along the RG and YB spectrum can provide a sensitive measure of the functional changes observed in DR. Their results showed almost equal RG and YB losses in both type I and type II diabetic subjects, there was no correlation to the duration of the disease and/or the absence of retinopathy. These findings agreed with other studies that suggested that chromatic sensitivity loss can precede structural changes in the retina (Hardy et al. 1992; Kurtenbach et al., 1999; Ong et al., 2003).

Although DR is diagnosed by visual fundus examination, it has become more evident as research progresses and more studies are conducted, that the functional changes taking place in the retina of a diabetic patient precede visible structural changes and that neuroretinal function is compromised before the vascular changes are observed. Hence CV may be good predictor for evaluation of such functional changes, allowing earlier intervention. When coupled with better diabetic control, the earlier intervention may reduce the rate of diabetes progression and related complications. The outcome is a better quality of life and reduced economic burden that accompanies the care for DR patients.

2.3.2 Colour vision tests

This section describes some of the CV tests and results reported in earlier studies. The latter were conducted mostly during the last decade and used a variety of CV tests to investigate the relationship between diabetes and CV loss. Other studies that aimed to detect neurodegenerative changes using alterations in the ERG will also be reviewed.

In the current study, chromatic sensitivity was measured using the Colour Assessment and Diagnosis (CAD) test which quantifies the severity of CV loss in a simple and effective way. The test enables accurate assessment of both RG and YB loss (Barbur and Rodriguez-Carmona, 2015) of chromatic sensitivity. The CAD test will be described in more details in section 3.2. The majority of other colour vision tests fail to quantify accurately the severity of colour vision loss and were designed mostly to screen for congenital colour deficiency.

2.3.2.1 Pseudoisochromatic test plates

The most common is the Ishihara colour screening test. It was first published in 1917; it is useful in screening for subjects with congenital colour deficiency (Belcher et al., 1958). The subject is presented with a series of colour plates with either numbers or convoluted lines embedded within a background of dots that vary in size, luminance and chromaticity. There are several editions available which contain 24 and 14 plates, however the standard edition contains 38 plates, 25 of which contain numerals (single or double-digit numbers), the remaining 13 plates are intended for examination of nonverbal subjects, where the subject is required to trace the pathway of convoluted lines. Normal trichomats will be able to see the correct numbers but an individual with colour deficiency will either fail to distinguish the number or will see different number. For example in hidden digits plate, normal trichomat will fail to see a number while a colour deficient subject might see a number. (Figure 2-2).

Errors made by CV deficient subjects are considered to be typical while errors made by normal trichomats are considered non-typical errors. Birch and

McKeever, 1993 described the non-typical errors made by normal trichomats as 'misreadings', it is attributed to the subject perceiving a loop of the serif as being complete rather than incomplete (for example, a '5' may be interpreted as a '6' or '3' as '8 '. The misreading is related to the strength of chromatic signal which means that the weaker the perceived chromatic signal in the test plate, the higher the probability of confusions or misreading (Rodriguez-Carmona et al., 2012).

4 or less errors are required for a normal trichomate to pass the test (Birch, 2001). Reducing the number of errors means increasing the sensitivity of the test but even then, if the pass score is set at 3 or less errors this will mean that 10% of deutans and 1%protans will also pass the test. If errors are not allowed, 19% of normal trichomats will fail the 38 plates edition with at least one error. This makes the outcome of the test not specific and it fails to give accurate measure of the severity of CV loss.

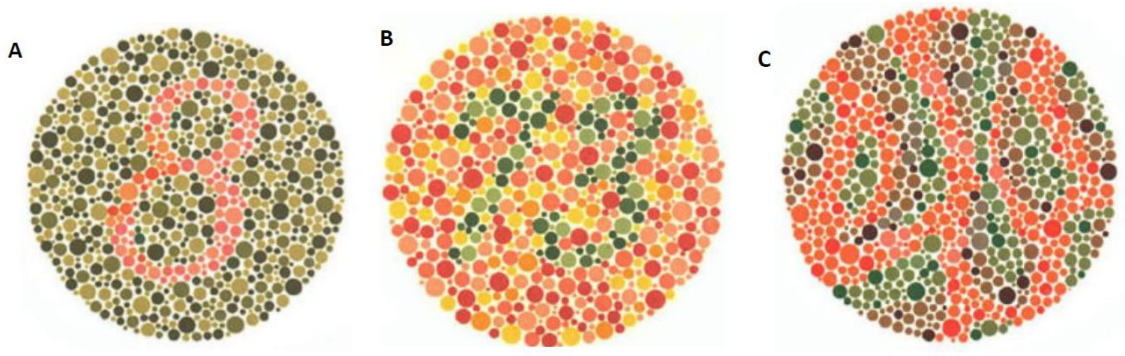


Figure 2-2: Illustration of the Ishihara Pseudoisochromatic plate test (38 plates edition). (A) Transformation plate, normal trichomats would see the number '8' while a colour deficient subject would see it as '3', (B) Vanishing plate, most colour deficient subjects would not see the number '73', (C) Hidden digit plate, normal trichomats would fail to see a number while a colour deficient subject might see a number '5'.

2.3.2.2 Arrangement tests

Arrangement tests utilize coloured caps with different hues. The subject is required to arrange the caps in order of hue. The test scores are reported in the form of error score that is derived from the number and pattern of errors made during the arrangement.

2.3.2.2.1 Farnsworth-Munsell Hue test

The FM-100 hue test aims to evaluate the subject's hue discrimination ability. The observer is required to arrange colour samples according to hue, lightness and saturation. There are 2 versions of the test, the Farnsworth-Munsell 100 (FM-100) hue test which has 85 colour samples and the Farnsworth-dichotomous test (D-15) which has 15 coloured samples. The Farnsworth-Munsell 100-Hue is formed of four 21 or 22-cap sets (Figure 2-3). The subject is instructed to start with the marked cap and arrange the rest in order according to their colour for each of the boxes. The test is scored by calculating error score and plotting graphical representation using the numbers on the back of the caps. Hue discrimination ability is measured as the square root of the total error score and the type of CV deficiency is established by interpreting a graphical illustration of the results (Figure 2-4). There is no direct correlation between severity of a colour defect and the error score (Birch, 1989). Subjects with mild or moderate colour deficiency would be able to pass the test due to the colour difference between adjacent caps (Birch, 2001). The performance on the test is influenced both by the illumination used as well as the skill, comprehension, and motivation of the subject (Farnsworth, 1957).

Figure 2-3: Illustration of the Farnsworth-Munsell 100 (FM-100) hue test.

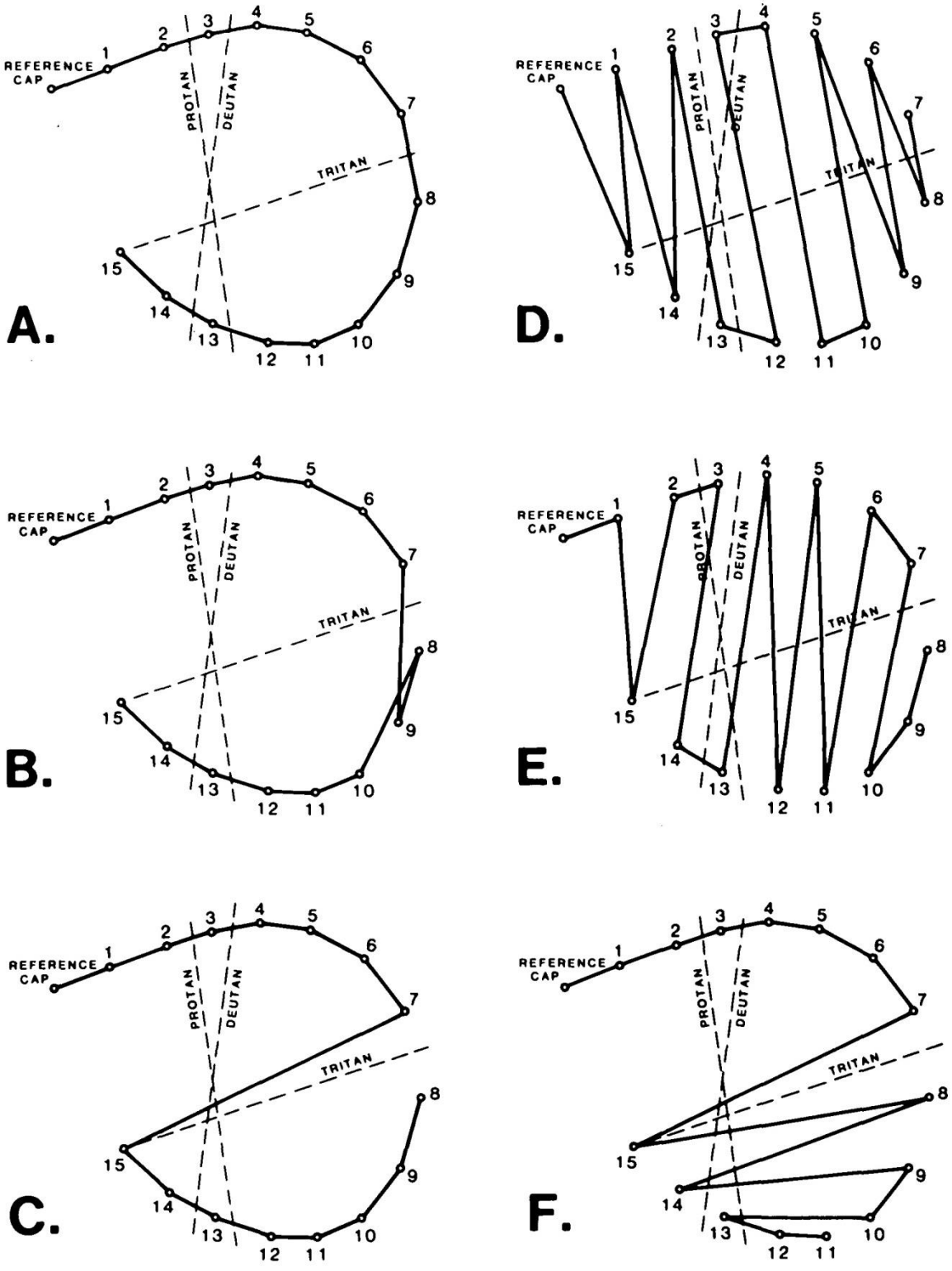


Figure 2-4: Graphical representation of the D-15 cap arrangements. (A), (B), (C): normal subjects, (D): a protan subject, (E): a deutan subject, (F): a tritan subject (adapted from Vingrys and King-Smith, 1988).

2.3.2.2.2 Lanthony's Desaturated Panel 15 test

This is another arrangement test; it has 15 colour samples which are less saturated than the Farnsworth Dichotomous test. This makes it more difficult to carry out. The test should be presented under high levels of illumination (> 500 lux). Rather than measuring the overall hue discrimination ability, it is used to detect moderate and severe colour deficiencies (Figure 2-5).

Figure 2-5: Illustration of the Lanthony's Desaturated Panel 15 test.

2.3.2.2.3 Lanthony New Colour Test

This test is intended to distinguish between mild, moderate and severe colour deficiency. It is formed of 70 Munsell samples, four series of 15 colours and 10 grey caps. The observer is required to arrange the coloured caps in colour order and the grey caps in order of lightness scale. Results are plotted on a graph and an error score is then calculated.

2.3.2.3 Nagel anomaloscope

The Rayleigh match was first described by Rayleigh (1881) and developed into an instrument for assessing red / green colour vision by Nagel in the early 1900's. It was designed for clinical evaluation of colour deficiency. The observer is presented by circular bipartite field subtending 3° in Maxwellian view, the lower hemi-field is illuminated with yellow light source, the luminance of which can be varied and the upper hemi-field is formed of mixture of adjustable red and green lights.

The test requires the observer to make colour matches by adjusting both the red-green ratio and the luminance of the yellow field. The observer is then required to determine whether adjustments in the luminance of the yellow field can or can't produce exact matches to red-green ratios that are set by the examiner. The type of colour deficiency is determined from the patterns of variation in the red/green mixture range and the intensity of the yellow field needed to match this variable mixture (Birch, 2001) (Figure 2-6).

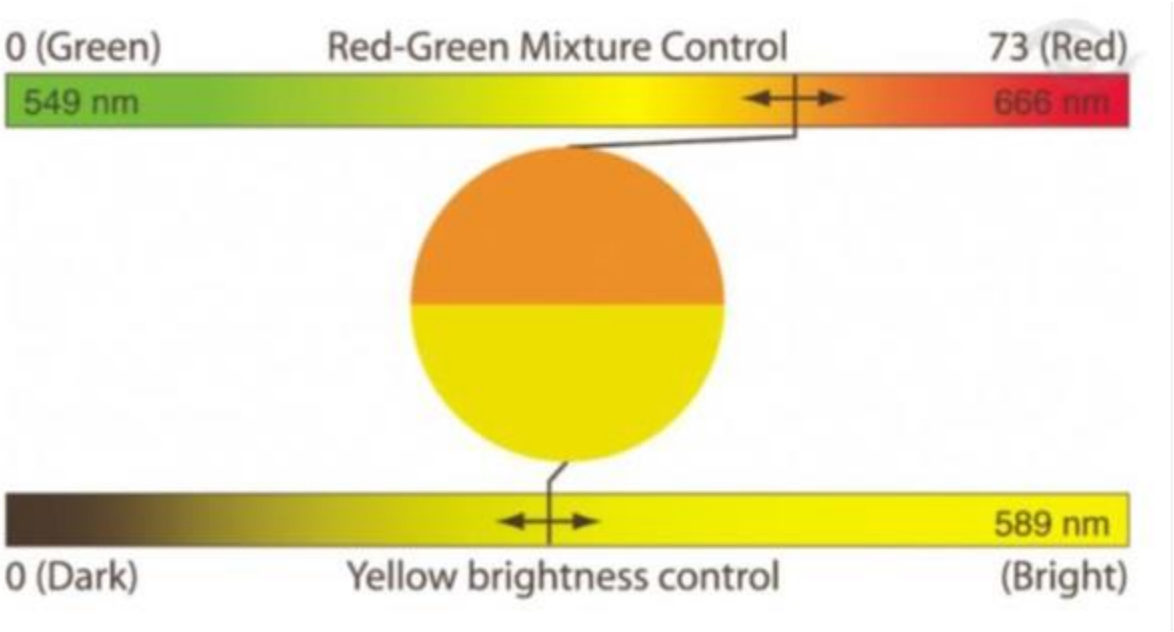


Figure 2-6: Illustration of the Nagel anomaloscope and the bipartite field. The upper hemi-field is formed from mixture of monochromatic red and monochromatic green and the lower hemi-field is illuminated with monochromatic yellow light source (adapted from Schiefer et al., 2007).

The ratio of red and green is quantified; this ratio is between 0 for monochromatic green to 73 for monochromatic red. Subjects with normal colour vision will typically be able to make a Rayleigh match when the proportion of the two lights are nearly equal (i.e.; in the range of 35 - 40). In a protan subject, they will need to increase the proportion of red light in the mixture in order to make a match. So protans will be able to match range of mixtures between 40 and 73. A deutan subject will require greater proportion of green light achieving the mixture range between 0 and 40.

The accuracy of diagnosis requires interpretation of the result by an experienced examiner to be able to interpret whether the subject has made a match or not based on their responses. In a study by Barbur et al., (2008) subjects with known genetic congenital colour deficiency still managed to make a Rayleigh match

within the normal range (Barbur et al., 2008). This means that the Nagel anomalscope is not as perfect instrument, as previously thought.

2.3.2.4 Automated / computerized tests

Some of these tests are based on the principals of the arrangements tests and others are based on colour contrast sensitivity. Examples of these tests include the Sussex Gratings Machine (SGM), the Chroma test and the Cambridge colour test.

2.3.3 Electroretinography

Several studies were conducted to investigate the neuroretinal changes using either full field or multifocal ERG. Those studies describe delayed or declined oscillatory potentials which are produced by the amacrine cells (Wachtmeister at al., 1978). Holopigian et al., (1997) investigated group of 12 diabetic patients with duration of diabetes more than 5 years. They detected delay in the a and b waves denoting changes in the photoreceptor cells. Similar results were shown by Fortune et al., (1999) they examined patients with 10-15 years duration of diabetes using multifocal ERG, they reported delay in the implicit time.

2.3.4 Microperimetry

It is used to measure retinal sensitivity. Several studies investigated the relationship between structural changed occurring within retinal layers in

diabetic patients and the effect that this would have on retinal sensitivity. As in recent study by Montesano et al., (2017), where they compared the retinal sensitivity to ganglion cell layer-inner plexiform layer thickness in 35 normal subjects and 26 diabetic subjects with no DR. They detected significant correlation between retinal sensitivity and the correspondent GCL-IPL thickness in diabetic subjects (0.022±0.006 dB/µm, p=0.0007) but not in healthy subjects (-0.002±0.006 dB/µm, p=0.77).

In another recent study by Nerianuri et al., (2017) they detected reduction in retinal senstivity measured by microperimetry in 245 subjects with no DR. Where mean retinal sensitivity was 14.78 ± 3.17 dB in subjects with absent diabetic neuropathy (DN) compared to 13.56 ± 3.65 dB in subjects with DN. This indicates the presence of neuroretinal dysfunction even in the absence of DR and supports the theory that neuronal changes precede the onset of vascular changes.

2.3.5 Contrast sensitivity

Contrast sensitivity (CS) is known to be affected in diabetic patients even in absence of DR (Della Sala et. al., 1985) (Di Leo et al., 1992). It was demonstrated in recent study by safi et al., (2017) they compared CS in 46 diabetic subjects with no DR to 46 normal control subjects. They measured CS at four spatial frequencies (3, 6, 12, 18 cycles per degree) under moderate (500 lux) and dim (less than 2 lux) background light conditions. CS was impaired in diabetic subjects at an early stage, before any clinical signs of retinopathy were detected. Nerianuri et al., (2017) measured CS in 613 subjects with no DR, CS was 1.36 ±

0.17 log units in absence of DN compared to 1.25 ± 0.21 log units in subjects with DN.

2.3.6 Aim of study

This study was set up to investigate the pattern of RG and YB CV loss in diabetic patients. In particular, we wanted to investigate the advantages of using the CAD test to detect and quantify severity of CV loss in patients with diabetes. The correlation with other diabetes-related factors such as type, grade, control and duration will be examined. The ability of the CAD test to detect early loss of colour vision that precedes other functional changes will also be investigated.

3 METHODS

This chapter describes the criteria and recruitment process of the subjects, assessment of the grade of DR and the tests that were used for measuring chromatic sensitivity and retinal thickness as well as a list of other patient-specific information that was collected such as duration and control of diabetes, HbA1C and previous medical and ophthalmic histories.

3.1 SUBJECTS

Subjects were recruited from the Ophthalmology outpatient department of King's College Hospital NHS Foundation Trust, London. The study was approved by the Integrated Research Application System (IRAS) (Ref:11/NW/0753), King's College Hospital Research and Ethics Committee as well as City, University of London Research and Ethics Committee. The study adhered to the principles of the declaration of Helsinki. Each subject was provided with an information sheet prior to their agreement to participate and was required to provide informed signed consent before taking part.

Only subjects who met the inclusion criteria were recruited into the study. The inclusion criteria for the study required that all subjects:

- Were aged 35 years or above, of either sex. Type II diabetes is more common after the age of 40-45 years and Type I is rare after that age (www.nhs.uk), so we set age 35 or above as an inclusion criteria to avoid a wide variation in age group between type I and Type II diabetics.
- Were able to provide informed consent.
- Had established diagnosis of Diabetes (Type I or II).

- Had no other ocular disease apart from DR.
- Had normal CV (subjects with congenital CV loss as assessed by the CAD test were excluded from our analysis).
- None of the subjects included in this study were taking medication known to affect CV, e.g. Digoxin (Lawrenson et al., 2002), ethambutol, chloroquine, hydroxychloroquine, phenytoin or sildenafil (Santaella and Fraunfelder, 2007).

The RG congenital colour deficiency affects about 8% of men and about 0.4% of women (Birch, 2001) and therefore we can expect at least 1 female and 5 males from our recruited subjects to have congenital RG deficiency. The reported average prevalence of colour blindness in the United Kingdom is 4.7% (www.nhs.uk). Initially subjects were asked about family history of CV loss and were considered to be normal if the answer was negative. However, after examining the results from the CAD test, we were able to detect 4 subjects with congenital CV loss, out of the 106 recruited (3.77%). Those are the subjects who exhibit RG losses whilst maintaining normal YB thresholds. Those subjects were excluded from the analysis.

In addition, subjects were excluded from the study if:

- Their best spectacle corrected visual acuity (BSCVA) was worse than 6/12 (0.3 LogMAR).
- They suffered from any other disease or had any other eye disease which could affect CV or mask the identification of the CAD test screen (e.g. multiple sclerosis and optic neuritis) (Russell et al., 1991).
- Any ocular condition in the study eye that could affect the results of the test, such as dense cataract of the crystalline lens or intraocular pressure ≥ 21 mmHg.

The density of cataract was graded using Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993) (appendix C), any patient with NO3, NC3, C3 or P2 or more was excluded.

The above inclusion / exclusion criteria were designed to enable the investigation of chromatic sensitivity threshold losses which are most likely to be caused by diabetes rather than any other, potentially confounding factors. Although the CAD test is least dependent on visual acuity, but usually poor visual acuity implies advanced retinal pathology with significant structural changes at the fovea. By recruiting subjects with 6/12 or better acuity, we ensure that CAD thresholds are not affected by other factors and that any changes in CV are likely to be the result of diabetes.

Subjects were recruited from the routine retinal clinics at King's College Hospital NHS Foundation Trust, this meant that assessment of presenting visual acuity was measured monocularly for distance only using Snellen's chart starting with the right eye and followed by the left eye. This was carried out by qualified nurses. For purpose of analysis, the measured values were transformed to LogMAR units using a calculator obtained from http://www.myvisiontest.com/logmar.php. Following the measurement of presenting visual acuity, subjects had their pupils dilated by instilling Tropicamide 1% eye drops in both eyes. Pupil size does not affect colour thresholds (Barbur et al. 1997; Barbur and Rodriguez-Carmona, 2012), also the background luminance and stimulus size employed in the CAD test are set to allow for variation in pupil size and viewing distance (Barbur and Rodriguez-Carmona, 2015) giving confidence that any variations in CV were not a result of variation in pupil size.

3.2 OPHTHALMOLOGICAL EXAMINATION

Full clinical ophthalmic examination was performed and this included taking medical and ophthalmic history together with other patient-specific information such as duration, control of diabetes and HbA1c. Subjects were then examined using a slit lamp for biomicroscope, followed by dilated fundus examination using non-contact 90D and digital wide field Volk® lenses. Intraocular pressure (IOP) was measured using Goldmann's applanation tonometry. At this stage, subjects were asked to join the study and those who were willing to take part, were given an information leaflet about the study and invited to sign the consent form (appendix A and B). 110 out of 115 subjects agreed to take part in our study (95.6% acceptance).

3.3 OPHTHALMIC INVESTIGATIONS AND IMAGING

All subjects had OCT scans using the standard protocol on the Heidelberg Spectralis SD-OCT (Heidelberg engineering GmbH, Heidelberg, Germany) (see section 3.3.2 for details of procedure). All scans were performed by two experienced medical photographers.

Subjects who were referred as new patients from the Diabetic Screening Service (DSS) had their fundus photos taken at screening and these were available for the study. Other subjects who were seen in clinics as follow-ups had their fundus photos taken specifically for this study by an experienced medical photographer. Photos were saved for future referencing and grading purposes.

This was followed by monocular measurements of chromatic sensitivity, using the CAD test (Barbur et al, 1994; Barbur and Connolly, 2011).

DR was graded according to ETDRS severity scale (discussed previously in section 2.2) as: no retinopathy, mild, moderate, severe DR, presence/absence of MO, and active or treated PDR.

3.3.1 Chromatic sensitivity

The standard CAD test measures chromatic sensitivity along 16 hue directions in the Commission Internationale d'Eclairage (CIE) 1931 - (x,y) chromaticity chart. The directions selected are grouped together to assess red-green and yellow-blue chromatic sensitivity.

The stimulus is generated with 30 bit resolution in the centre of a large uniform background field on a visual display (La CIE Electron Blue, 20" CRT monitor). The stimulus consists of a square array of 15 x 15 achromatic checks, subtending a horizontal visual angle of approximately 3.3° x 3.3° at a viewing distance of 1.4m. The background chromaticity is set as the MacAdam 'white' in the 1931 CIE – (x,y) chromaticity coordinates: x=0.305, y=0.323. The luminance of each checker scintillates every 40 to 80 ms, with equal probability, within a range defined as a percentage of background luminance; the standard CAD recipe employs dynamic noise amplitude of ± 45%. The variation of the checks is such that the average check luminance over time and the average spatial luminance of the stimulus remain unchanged and equal to that of the background, thus providing a steady state of light adaptation independently of any chromatic displacement.

This random luminance modulation is applied spatially and temporally, creating achromatic dynamic luminance contrast (LC) noise that masks the detection of luminance contrast signals and isolates the use of chromatic signals (Barbur and Ruddock, 1980; Barbur et al., 1981; Barbur et al., 1994; Rodriguez-Carmona et al., 2005).

There is a smaller, colour-defined, square outline (comprising 5 x 5 checks and subtending a visual angle ~ 0.8° at a viewing distance of 1.4 m) buried in the larger array of LC checks. The test stimulus moves at a speed of ~ 4°/s along the diagonals of the larger square defined by LC noise (Figure 3-1). An efficient, four-alternative, forced-choice procedure is used to measure the subject's chromatic displacement thresholds, the subject's task is to press one of four buttons arranged at the corners of a square on a wireless response control, following each stimulus presentation to indicate the direction of motion of the colour defined stimulus. Each presentation is followed by a brief audio cue to prompt the subject to make a response. The correct direction has to be reported twice in succession before the colour signal of the stimulus is decreased, according to the interleaved staircase procedure making the chance probability of a correct response 1 in 16 (Barbur, 2004).

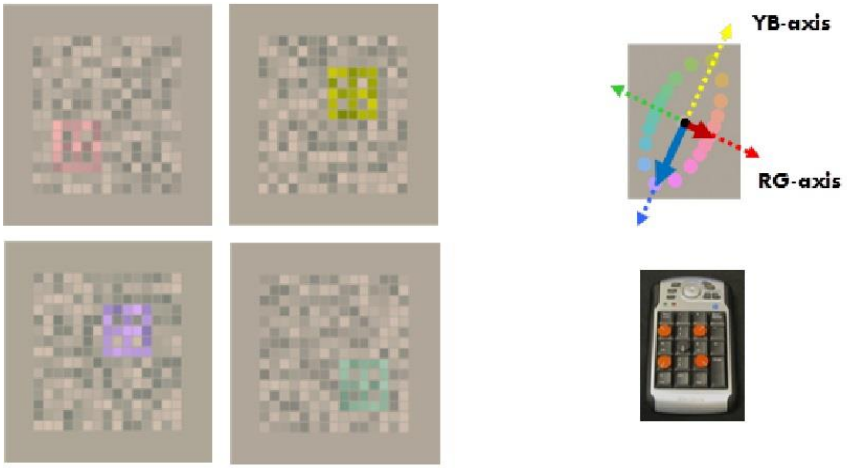


Figure 3-1: The appearance of the CAD test moving coloured stimuli during the test together with the numeric keypad used to record the subject's responses. The colours shown correspond to the RG and YB isolating axes.

The CAD test algorithm averages the thresholds measured for some of the hue directions investigated to compute the mean RG and YB thresholds. Subjects with refractive errors wore their appropriate refractive correction for the corresponding distance.

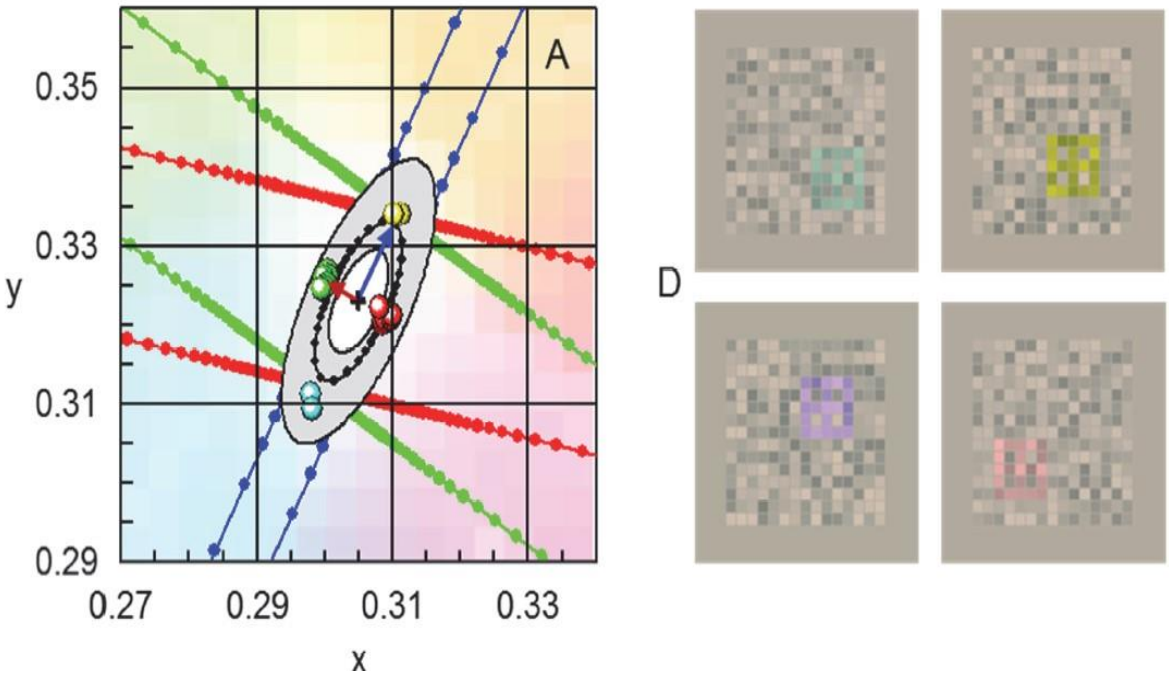


Figure 3-2: CAD test result for typical, normal trichromat plotted in the CIE 1931 (x,y) chromaticity chart. The black cross at the centre of the diagram plots the chromaticity of the white background i.e., 0.305, 0.323. Grey shaded area shows the range of variation expected in normal CV at the corresponding age (see Fig. 3.3). The red and blue arrows represent 1 CAD unit for RG and YB, respectively. The normal, upper threshold limits have been measured as a function age and are used by the CAD test to decide whether the subject's RG and YB thresholds are within the normal range (Barbur & Rodriguez-Carmona, 2015).

All subjects were positioned 1.4 m away from the visual display with the eye horizontally in line with the centre of the monitor, the test was carried out monocularly in the eye with the best VA. The 'definitive' CAD test takes ~ 8 minutes to assess RG and ~ 4 minutes to assess YB thresholds. The 'learning mode' required 100% correct responses to ensure that the subject understood the testing procedure and the use of the numeric keypad (see Fig. 3.1).

The initial validation of the CAD test and the statistical limits for the normal CAD observer is based on RG and YB thresholds measured in 330 young, healthy subjects with normal trichromatic colour vision (Civil Aviation Authority, 2009).

Figure 3-2 shows the CAD template plot for a normal trichomat , where the grey shaded area shows the range of variation expected in normal CV, the innermost and outermost ellipses corresponding to 2.5% and 97.5% confidence limits for normal population, respectively (Rodriguez-Carmona et al., 2005). The dotted black ellipse represents median value for the standard normal trichromatic; this is expressed in 'standard normal units' (SNU) which is taken as 1 SNU.

3.3.1.1 Age related changes in colour vision

Many changes that take place during the aging process will affect CV, although this change is gradual and can often go unnoticed for several years. The first of these changes takes place in the pre-receptoral filters of the eye, the most important of which, is the crystalline lens. The lens acts as a filter for the transmittance of short wavelength light, preventing wavelength shorter than 300 nm from reaching the retina. The lens has high optical density (OD) that decreases rapidly above 450 nm allowing the transmission of incident light for wavelengths longer than 560 nm (Norren and Vos, 1974). Aging and increased absorption of short wavelength light cause the yellowish appearance of the lens (Pokorny et al., 1987; Weale, 1988). This reduction in transmission affects the retinal illuminance and affects mainly short wavelength light. This contributes to the more rapid loss of YB chromatic sensitivity seen with aging (Barbur and Rodriguez-Carmona, 2015).

The macular pigment optical density (MPOD) also affects the absorption of light. It is yellow in appearance and absorbs light in the blue range of visible spectrum with peak absorption at ~ 460 nm (Bone et al., 1992). Age related changes at the macula have been identified as an underlying cause of reduced chromatic sensitivity (O'Neill-Biba et al., 2010).

The linear loss of retinal ganglion cell (RGC) axons and cell bodies is another important factor contributing for increased RG and YB thresholds observed in normal aging. There is about 40% linear reduction in RGC axons during the life span (Jonas et al., 1992; Neufeld and Gachie, 2003; Calkins, 2013). The rod photoreceptor density reduces but the number of cones remains relatively constant in normal ageing (Curcio et al., 1993).

Barbur and Rodriguez-Carmona (2015), studied the effect normal aging has on RG and YB thresholds, as measured with the CAD test. They found that the optimum age for best colour thresholds was around ~ 20 years of age, this was followed by gradual and linear increase of ~1% per year for RG and ~1.6% for YB thresholds over the remaining life span (Figure 3-3). The mean monocular thresholds as a function of age are given by the following equation:

RG_{mon} = 0.901 + 0.0156*age +4.351*exp(-0.19*age) and

YB_{mon} = 0.306 + 0.0283*age +3.889*exp(-0.1136*age).

Then to evaluate the upper normal limit ($\pm 2.5\sigma$) one must add 0.993 to RG and 1.027 to YB.

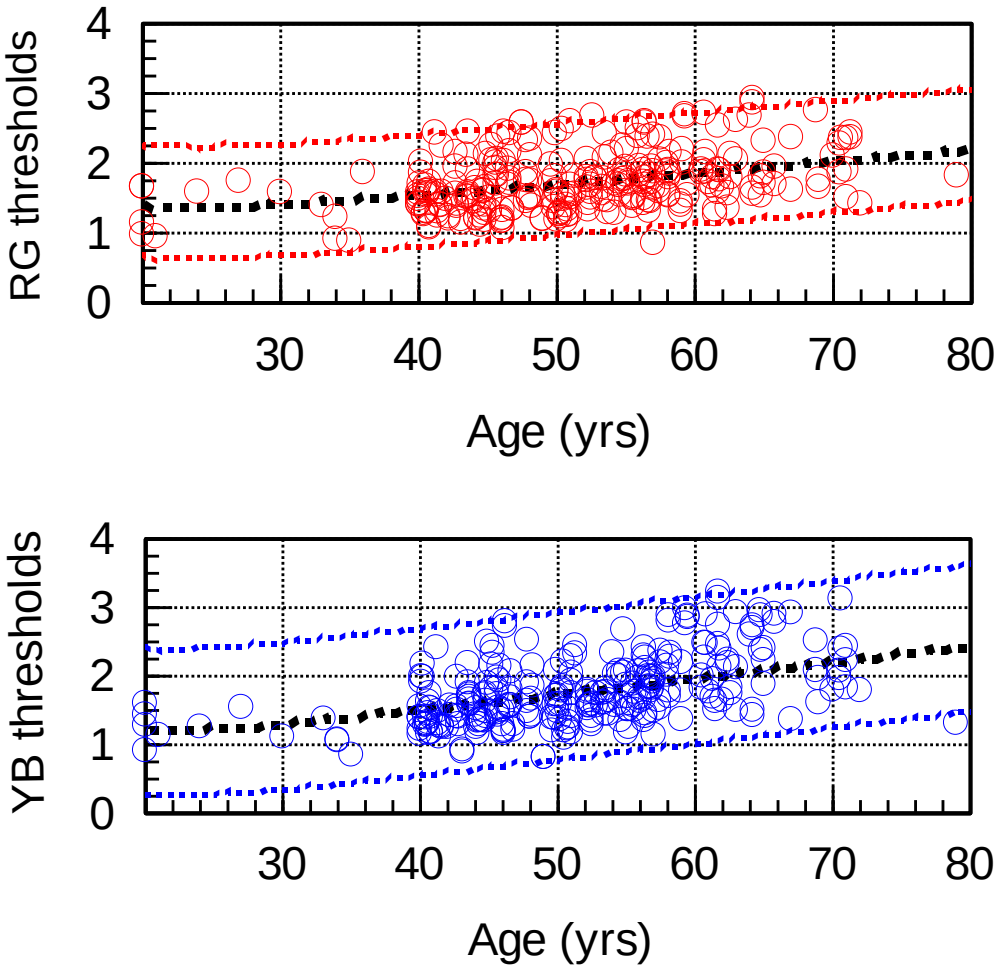


Figure 3-3: Normal trichomats RG and YB thresholds for age. A graph showing the RG and YB thresholds as a function of age for normal trichomats subjects (Barbur and Rodriguez-Carmona, 2015).

3.3.2 Optical coherence tomography

OCT has established itself as valuable and some might argue essential method in the evaluation and management of retinal diseases in general and macular diseases in particular (Drexler and Fujimoto, 2008). It has allowed virtual visualization of the human retina at high resolution and given a new aspect in the interpretation of clinical findings. Since it was introduced in the late 1990s, it has evolved hugely reaching a resolution which allows visualisation of retinal

details down to 10µm. This has helped in better understanding of pathologies of various retinal diseases and has greatly improved clinical diagnosis and increased current knowledge of DR, in particular with detection and qualification of MO and modified current management approaches.

OCT scans were carried out using the standard protocol on the Heidelberg Spectralis SD-OCT. All scans were performed by two experienced medical photographers. The subject was asked to fixate on a stimulus, a cross green segments arranged like a star. During the examination, the patient was able to see the stimulus and a red scanning segment moving along it at the same time. During the scan the operator is able to visualise an external view of the eye, real time fundus image and the OCT images of the macula, after the capture of the scan qualitative information is shown on the review screen and any scan with quality signal ≤7 is disregarded. The Spectralis OCT camera uses an internal fixation source that tracks the subject's fovea and an infrared camera through which the stability of fixation can be monitored by the operator. The cross-sectional images are then analysed using incorporated software that is capable of mapping the strongest two edges in each tomogram that present the vitreoretinal interface and the basement membrane of the retinal pigment epithelial (RPE) – Bruch's membrane; respectively. Retinal thickness (RT) measurements are generated automatically and were obtained in 9 subfields (Figure 3-4 and 3-5) and described in the ETDRS. These subfields are arranged in inner, intermediate and outer rings with radii of 1mm, 2.22mm and 3.45mm respectively. The average of all points within the inner circle is defined as central sub-field thickness (CSF).

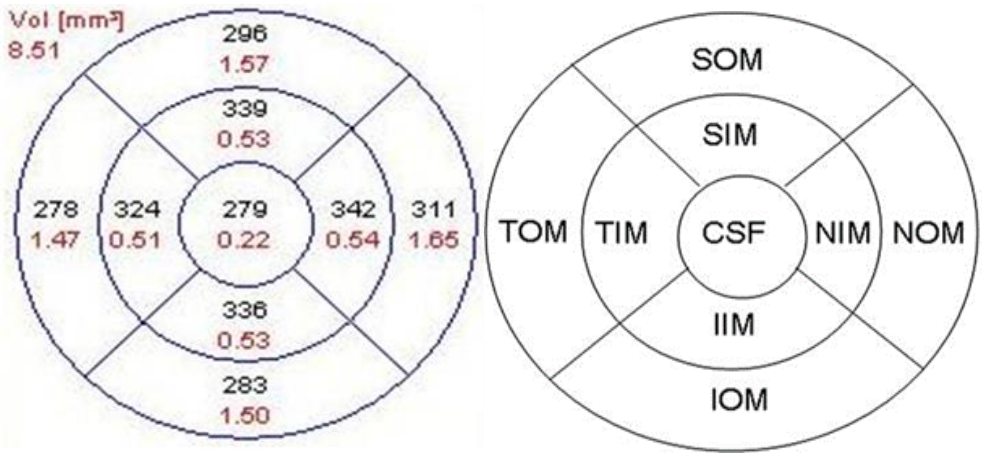
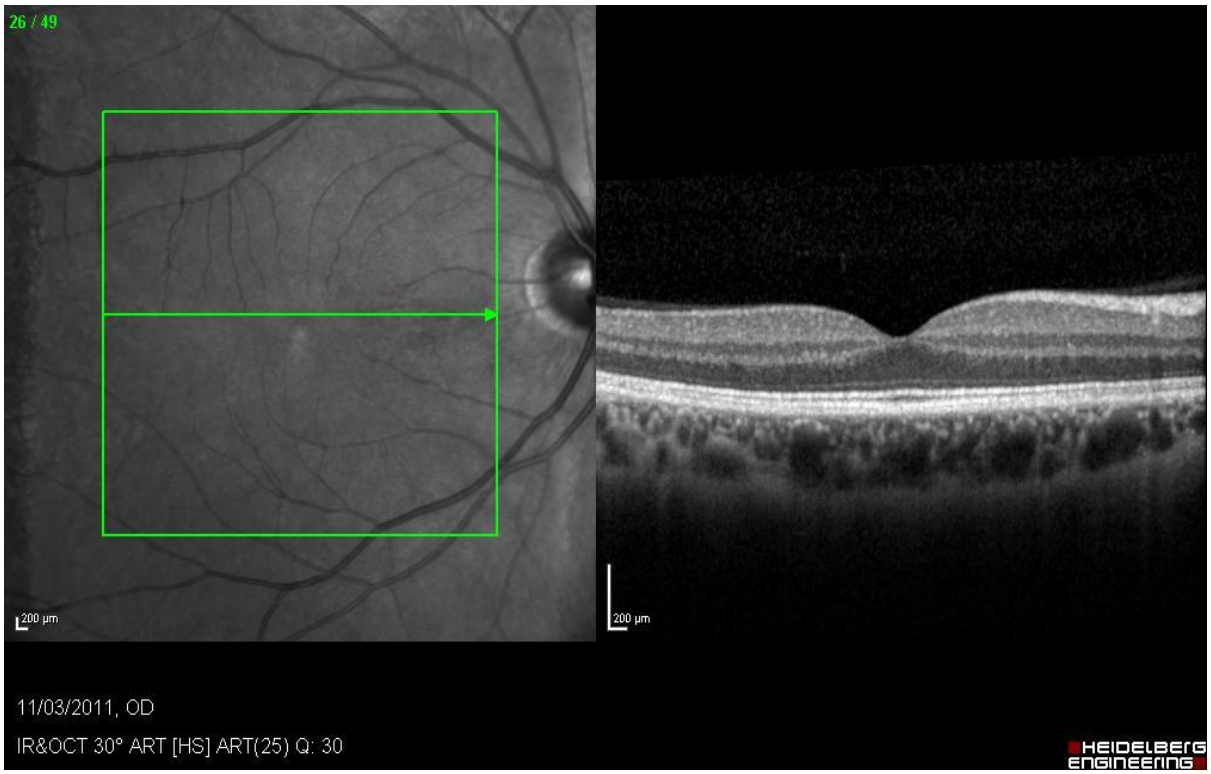


Figure 3-4: The standard 9 ETDRS subfields (Right) with their corresponding overall mean RT measurements (Left). CSF= central subfield; IIM= inferior inner macula; IOM= inferior outer macula; NIM= nasal inner macula; NOM= nasal outer macula; SIM= superior inner macula; SOM= superior outer macula; TIM= temporal inner macula; TOM= temporal outer macula.



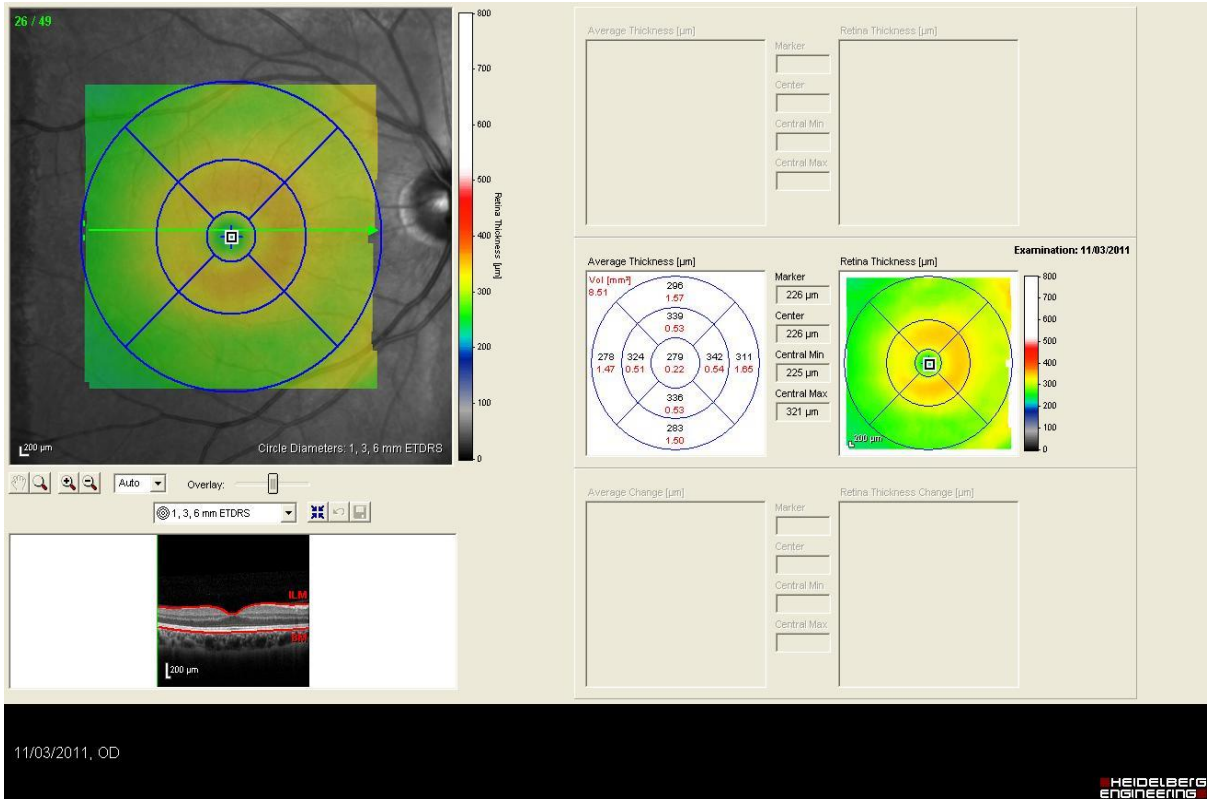


Figure 3-5: The appearance of Heidelberg Spectralis SD-OCT, scan showing normal right eye with retinal thickness measurements obtained in the 9 ETDRS subfields, including central subfield (CSF).

3.4 STATISTICAL ANALYSIS

The data were analysed using Microsoft Excel (MS Excel 2013, Seattle, WA, USA) and SPSS programs (SPSS V.22.0, SPSS, Chicago, IL, USA). Analysis was performed using t-tests to calculate p values and data were assumed significant if $p<0.05$. Categorical variables were examined using Pearson's r correlation.

4 RESULTS

4.1 SUBJECTS

A total of 106 subjects were recruited according to the inclusion criteria outlined in section 3.1. All subjects were assessed for RG and YB CV using the CAD test, as described in section 3.4. Only 102 subjects were included in the analysis as the CAD test results identified 4 subjects with congenital RG colour deficiency. This is usually the case when subjects exhibit RG losses whilst maintaining normal YB thresholds.

8% and 0.4% of healthy males and females, respectively have abnormal cone pigments and exhibit RG colour deficiency (Birch 2001) with the average prevalence of colour blindness in the United Kingdom estimated to be 4.7% (www.nhs.uk) which matches our findings as 4 out of 106 subjects (3.77%) had congenital colour deficiency.

4.1.1 Acquired causes for colour vision loss:

4.1.1.1 Lens opacity

9 out of 102 subjects (9%) were pseudophakic, the remaining had variable degrees of lens opacification. The density of lens opacity was graded using Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993) (Appendix C). This system uses a photographic set of images to illustrate different grades of nuclear, cortical and posterior subcapsular lens opacities. The grades range from

1 to 4, nuclear opalescence grade of NO1–NO2 or a nuclear colour grade of NC1–NC2 (Table 4-1).

Subjects included in our study were graded as follows:

Grade	No. of subjects
N01	77
N01C1	10
N01C2	2
N02	1
N02C2	2
P1	1

Table 4-1: Grades of lens opacities in subjects recruited in the study.

The increase in lens opacity causes a decrease in the transmittance of short wavelength light which in turn leads to reduction in retinal illuminance. This effect may contribute to the more rapid loss of YB chromatic sensitivity seen with aging (Barbur and Rodriguez-Carmona, 2015). It was therefore important to consider this, as this could potentially be a confounding factor in our analysis. We therefore analysed our results after matching for age groups and considering the age related upper threshold limits to enable us to separate the more gradual RG and YB losses which are attributable to normal aging from the diabetes induced changes. 18 subjects had RG thresholds below the corresponding, age-matched, upper normal limit, but only 4 of the subjects had YB thresholds within the normal range. All other subjects had RG and YB thresholds higher than expected for their corresponding age. This loss was higher than the normal gradual loss seen with aging and most likely losses must be caused by other factors such as the diabetes induced changes.

4.1.1.2 The effect of age on chromatic sensitivity

We compared the RG and YB thresholds results to the age matched controls. Only 19% of the diabetic subjects (n=19) had colour thresholds within the normal age limits (Figure 4-1). The age matched values were obtained from previously available data published by Barbur et al., 2015, using data from 443 normal eyes for RG and 456 normal eyes for YB CV. The results provided mean monocular RG and YB thresholds and the corresponding ±2.5σ limits as a function of age.

Diabetics with either RG or YB monocular thresholds above normal limits

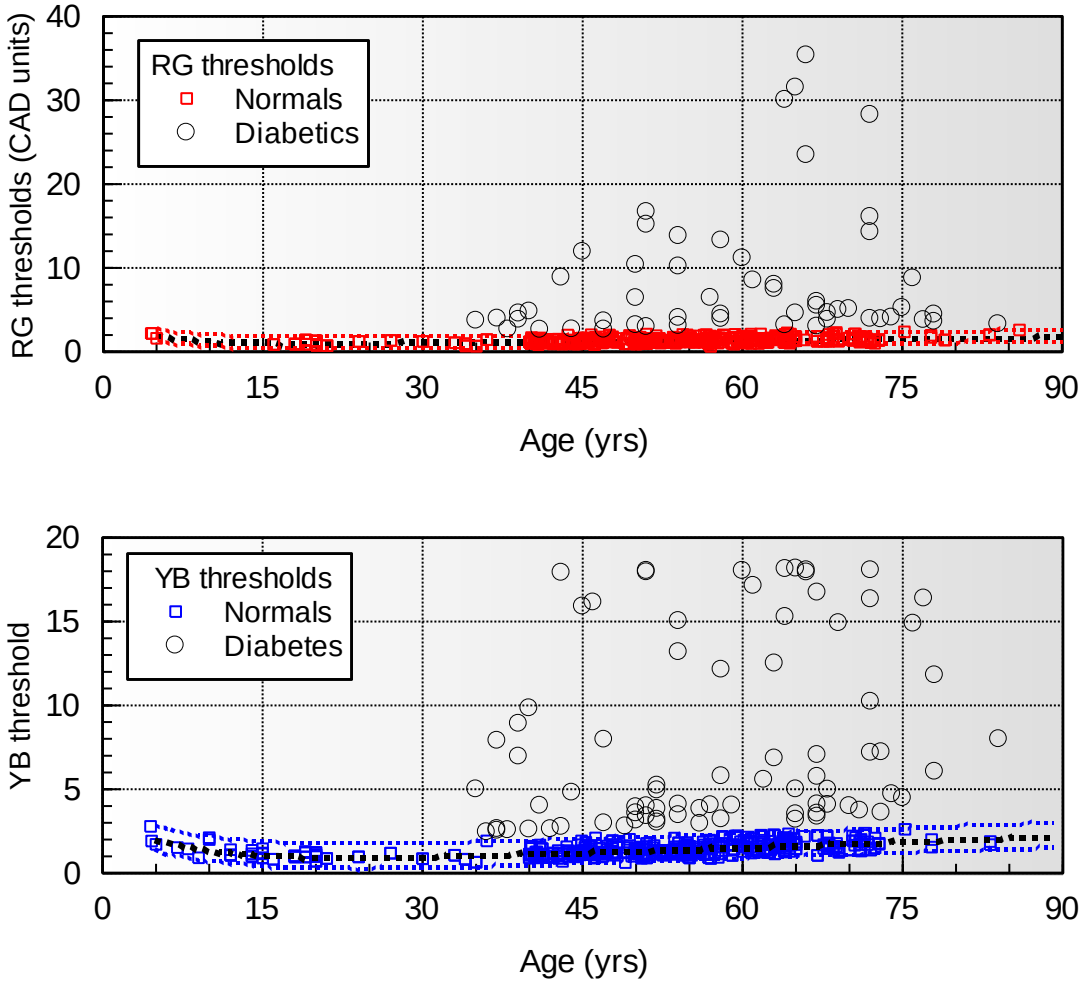


Figure 4-1: RG and YB thresholds measured in the diabetes group plotted as a function of age together with the corresponding data for normal, healthy trichromats (normative data based on 443 and 456 eyes for RG and YB, respectively which were filtered for congenital and acquired colour deficiencies). The results show that 82% and 96% subjects had RG and YB thresholds, above the corresponding normal upper age limits, respectively.

4.1.1.3 The effect of drugs on chromatic sensitivity:

As described in the inclusion and exclusion criteria in section 3.1, to our knowledge none of the subjects recruited in this study were taking medication known to affect CV.

4.2 ANALYSIS

The analysis included 102 subjects, 61 (60%) males and 41 (40%) females. Subjects were divided in 3 groups according to the severity of their DR. The 3 groups were; Group 1: No or mild NPDR, group 2: Moderate to severe NPDR and group 3: Active or treated PDR.

4.2.1 Group (1): No or mild NPDR

4.2.1.1 Descriptive data

This group included 67 subjects. The subjects demographic and baseline characteristics are shown in table 4-2.

Mean age (SD), years	56±12.14
Range, years	35-84
Gender, n (%)	
Male, *n* (%)	40 (60)
Female, *n* (%)	27 (40)
Race, n (%)	
Afro-Caribbean	40 (60)
Asian	5 (7)
Caucasian	22 (33)
Smokers, *n* (%)	1 (1.5)
Mean HbA1C, n (SD)*	8.14±1.55
≤8%, *n* (%)	25 (57)
>8%, *n* (%)	19 (43)
Type of diabetes, *n (%)*	
Type I	6 (9)
Type II	61 (91)
Control of diabetes, *n** (%)*	
Diet control	1 (1.5)
Metformin	36 (54)
Insulin	17 (25)
Insulin + Metformin	7 (10)
Mean duration of diabetes (SD), years	15.51±10.65
Range, years	1.5-40
Hypertensives on treatment, *n* (%)	32 (48)
Previous macular laser, *n* (%)	2 (3)
Vision in LogMAR,	
≤0.00, *n* (%)	61(91)
>0.00, *n* (%)	6 (9)

Abbreviations: HbA1C, glycosylated haemoglobin;
*n =44, as data is missing for 23 patients
** $n=61$, as data is missing for 23 patients

Table 4-2: Subjects demographic and baseline characteristics for group 1 (no or mild NPDR).

4.2.1.2 Change in RG and YB thresholds in group 1

17 (25%) subjects in this group had RG and YB thresholds within the normal limits, the remaining 50 (75%) subjects showed varying degrees of chromatic sensitivity loss. The mean RG and YB thresholds for the group as a whole were 3.69±3.95 and 4.92±4.21 SNU, respectively. The 50 subjects with RG and YB thresholds outside the normal limits had RG and YB threshold of 4.42±4.37 and 5.81±4.54 SNU, respectively. There were 43 subjects with RG thresholds <6, their mean thresholds were 3.20±1.33. Similarly, 34 subjects had YB thresholds <6, (3.26±1.03).

Most subjects (n=61) had BCVA 0.00 LogMAR or better except for 3 subjects who had LogMAR VAs of 0.08, 0.12 and 0.34. 2 out of the 3 subjects had undergone previous macular grid laser treatment.

In this group the $\mu\pm2\sigma$ central subfield thickness (CSF) as measured on OCT was 289±72 μm (range 214 to 362 μm).

4.2.2 Group (2): Moderate to severe NPDR

4.2.2.1 Descriptive data

This group included 22 subjects. The subjects demographic and baseline characteristics are shown in table 4-3.

Mean age (SD), years	55±11.98
Range, years	35-76
Gender, n (%)	
Male, *n* (%)	13 (59)
Female, *n* (%)	9 (41)
Race, n (%)	
Afro-Caribbean	9 (41)
Asian	0 (0)
Caucasian	13 (59)
Smokers*, n* (%)	3 (14)
Mean HbA1C, n (SD)*	7.66±0.92
≤8%, *n* (%)	9 (60)
>8%, *n* (%)	6 (40)
Type of diabetes, *n (%)*	
Type I	6 (27)
Type II	16 (73)
Control of diabetes, *n (%)*	
Diet control	1 (0.5)
Metformin	7 (32)
Insulin	8 (36)
Insulin + Metformin	6 (27)
Mean duration of diabetes (SD), years	20.77±11.18
Range, years	1-43
Hypertensives on treatment, *n* (%)	13 (59)
Previous macular laser, *n* (%)	3 (14)
Vision in LogMAR*,*	
≤0.00, *n* (%)	17 (77)
>0.00, *n* (%)	5 (23)

Abbreviations: HbA1C, glycosylated haemoglobin;
**n* =15, as data is missing for 7 patients

Table 4-3: Subjects demographic and baseline characteristics for group 2 (moderate and severe NPDR).

4.2.2.2 Change in RG and YB thresholds in group 2

Most subjects (n=20) in this group showed RG and YB losses above the normal age limits. The mean RG and YB thresholds for the group as a whole were 4.77±4.16 and 6.72±5.17 SNU, respectively. After excluding the 2 subjects with RG threshold within the normal age limit, the mean RG was 5.09±4.23 and YB was 7.10±5.27 SNU.

Most subjects (n=17) had BCVA 0.00 LogMAR or better except for 1 subject who had BCVA of 0.08 LogMAR and 4 other subjects who had BCVA of 0.18 LogMAR.

In this group the $\mu \pm 2\sigma$ CST as measured on OCT was 272±36 µm (range 215 to 344 µm).

4.2.3 Group (3): Active or treated PDR

4.2.3.1 Descriptive data

This group included 13 subjects. The subjects demographic and baseline characteristics are shown in table 4-4.

Mean age (SD), years	54±12.31
Range, years	37-68
Gender, n (%)	
Male, *n* (%)	8 (62)
Female, *n* (%)	5 (38)
Race, n (%)	
Afro-Caribbean	4 (31)
Asian	0 (0)
Caucasian	9 (69)
Smokers, *n* (%)	2 (15)
Mean HbA1C, n (SD)*	8.02±1.01

≤8%, *n* (%)	4 (50)
>8%, *n* (%)	4 (50)
Type of diabetes, *n (%)*	
Type I	7 (54)
Type II	6 (46)
Control of diabetes, *n (%)*	
Diet control	0 (0)
Metformin	2 (15)
Insulin	8 (66)
Insulin + Metformin	3 (23)
Mean duration of diabetes (SD), years	20.5±14.85
Range, years	2-56
Hypertensives on treatment, *n* (%)	9 (69)
Previous macular laser, *n* (%)	6 (46)
Previous PRP laser, *n* (%)	9 (69)
Vision in LogMAR,	
≤0.00, *n* (%)	7 (54)
>0.00, *n* (%)	6 (46)

Abbreviations: HbA1C, glycosylated haemoglobin; PRP. Pan retinal photocoagulation
**n* =8, as data are missing for 5 patients

Table 4-4: Subjects demographic and baseline characteristics for group 3 (Active or treated PDR).

4.2.3.2 Change in RG and YB thresholds in group 3

All subjects (n=13) showed RG and YB losses above the normal age limits. The mean RG and YB thresholds were 9.70±9.05 and 11.45±6.40 SNU, respectively (table 4-5).

7 subjects had BCVA 0.00 LogMAR or better, 2 subjects had BCVA 0.04 LogMAR and remaining 4 subjects had BCVA ranging from 0.10-0.30 LogMAR.

In this group the μ±2σ CST as measured on OCT was 351±105 μm (range 232 to 629 μm).

Group number	Number of patients	RG threshold	YB threshold
Group 1	50	4.42 ± 4.37	5.81 ± 4.54
Group 2	20	5.09 ± 4.23	7.10 ± 5.27
Group 3	13	9.70 ± 9.05	11.45 ± 6.40

Table 4-5: Showing the RG and YB thresholds for subjects within the 3 groups who had thresholds outside the normal limits.

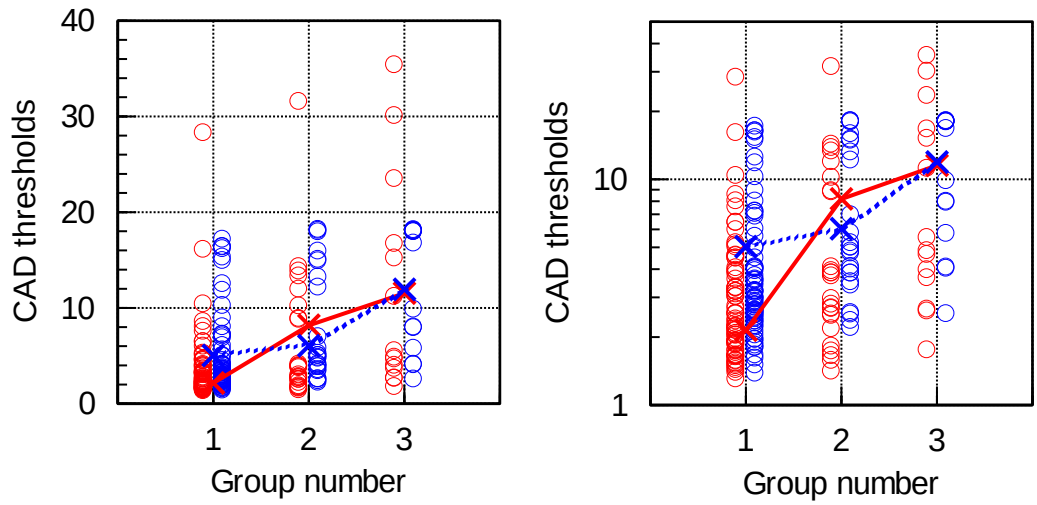
4.3 CHROMATIC SENSITIVITY AND DIABETES

4.3.1 Effect of grade of diabetic retinopathy on chromatic sensitivity

The measured RG and YB thresholds for the subjects that showed CV loss were variable, with some exhibiting RG losses greater than YB and others showing YB losses greater than RG.

The RG and YB losses were detected in the subjects irrespective of the presence or absence of DR. As seen with group 1, RG and YB losses were detected in subjects who had no or mild evidence of DR.

The results also show that RG and YB thresholds losses increase with the severity of DR as illustrated in figure 4-2. When comparing the RG thresholds between the three groups using analysis of variance (ANOVA), F-value= 8.11, while F crit. = 3.08 ($p<0.05$). As for the YB thresholds, F-value=10.49, F crit. = 3.08, but this was not significant. These results suggest that there is a greater loss of chromatic sensitivity in patients with more severe DR.



1= No or mild NPDR	
2= Moderate to severe NPDR	
3= Treated or active PDR	

Figure 4-2: RG and YB thresholds in three groups. Group 1: No or mild NPDR, group 2: Moderate to severe NPDR and group 3: Active or treated PDR. In group 1, RG and YB $\mu+2\sigma$ was 3.69±3.95 and 4.92±4.21, group 2 4.77±4.16 and 6.72±5.17 and group 3 was 9.70±9.05 and 11.45±6.40.

4.3.2 Effect of duration of diabetes on chromatic sensitivity

The mean±2σ of duration of diabetes in our subjects was 15.51±10.86 years with a range of 1-56 years. We examined the data to see if the duration of diabetes would show an effect on the RG and YB thresholds. No correlation was observed between RG and YB thresholds loss and the duration of diabetes with r^2= 0.0 and r^2= 0.01 respectively (Figure 4-3).

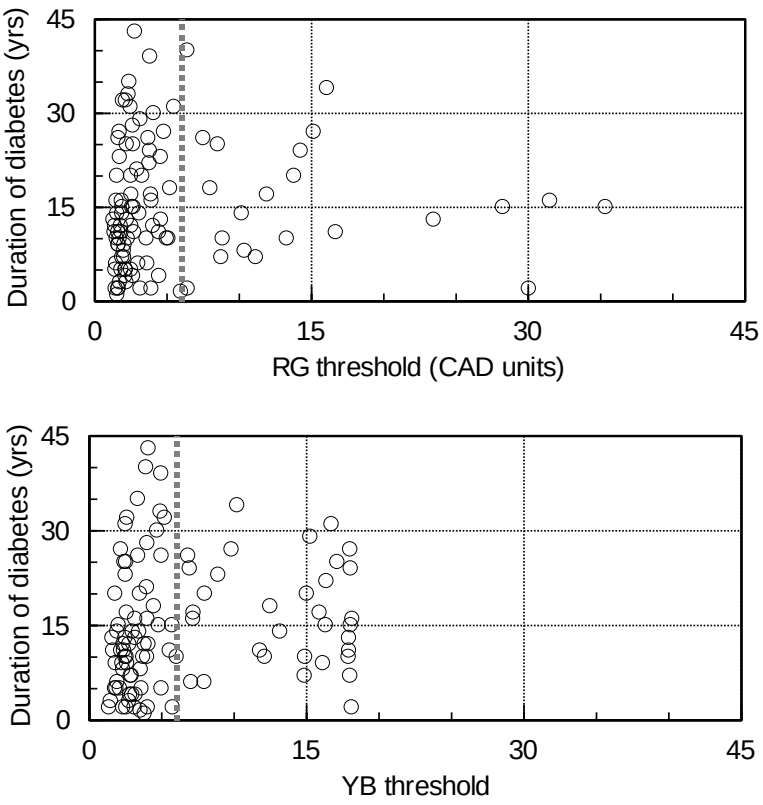


Figure 4-3: Effect of duration of diabetes on measured RG and YB thresholds. It was measured in 102 patients who had diabetes for a duration that ranged between 1-56 years, mean±2σ was 15.51±10.86 years.

4.3.3 Effect of control of diabetes on chromatic sensitivity

The control of diabetes included measurement of HbA1C levels. The mean±2σ was 8.13±1.56 %. This parameter did not correlate with losses of either RG or YB thresholds with r^2=0.02 and r^2=0.0 respectively. Another aspect of diabetes control is treatment; 47 (46%) subjects were using insulin alone or a combination of insulin and metformin, 45 (44%) subjects were using metformin alone and 2 (2%) subjects were only on diet control. Those two subjects were excluded from the analysis as it was a too small sample to assess any conclusive effect of diet control on the degree of CV loss. These data were not available for

8 (8%) subjects. Comparing the two groups, in the group that was controlled by either insulin or a combination of insulin and metformin the mean±2σ RG threshold was 5.00±5.65 SNU and YB threshold 6.49±5.11 SNU. The other group which had subjects controlled by metformin alone, the mean±2σ RG threshold was 4.91±5.19 SNU and YB threshold 6.41±5.48 SNU respectively (Figure 4-4). The mean of control of diabetes, whether it is insulin or metformin or both together didn't seem to have an effect on the chromatic sensitivity levels (p=0.93 and 0.97).

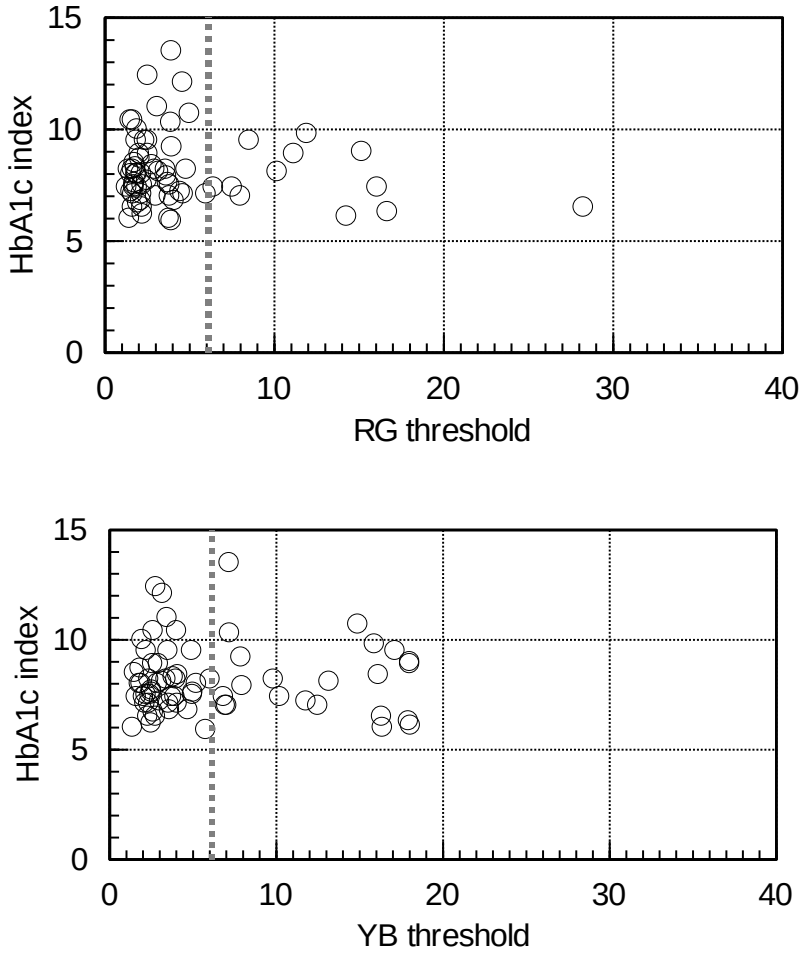


Figure 4-4: Effect of glycaemic control on measured RG and YB thresholds. HbA1C was measured in 67 subjects across the three different groups with mean±2σ 8.13±1.56 %. The control did not correlate with losses of either RG or YB thresholds.

4.3.4 Effect of Type of diabetes on chromatic sensitivity

20 subjects (20%) were type 1 diabetes with mean±2σ RG threshold 4.48±4.94 SNU and YB threshold 6.02±5.08 SNU. The other 82 subjects (80%) were type 2, their RG and YB thresholds were 4.70±5.25 and 6.14±5.17 SNU, respectively (Figure 4-5).

No correlation was found between the type of diabetes and the severity of loss in RG and YB thresholds with r^2=0.0 and r^2= 0.0 respectively.

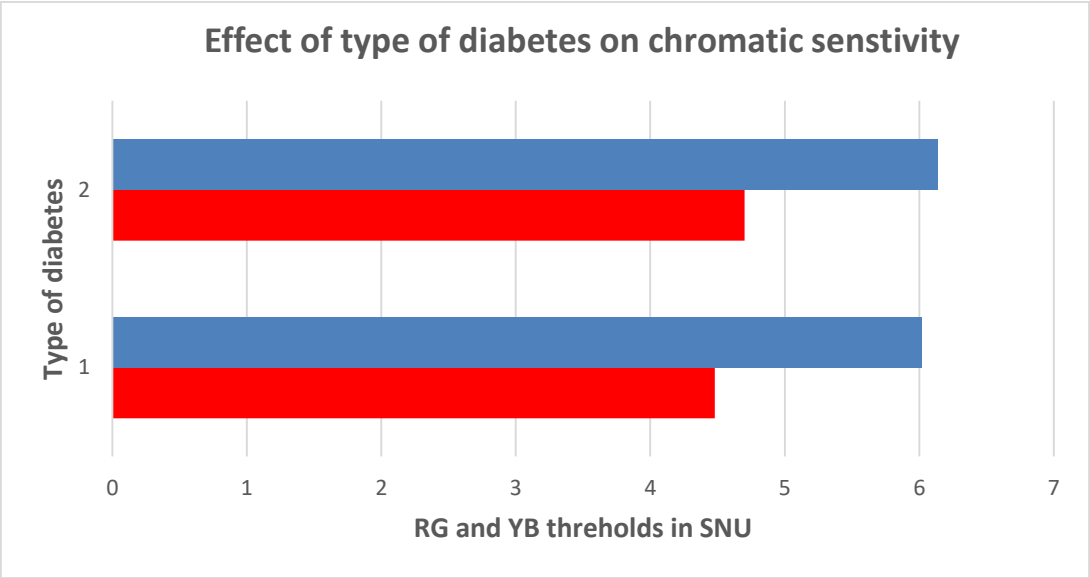


Figure 4-5: Effect of type of diabetes on chromatic sensitivity. The severity of colour loss was nearly equal in both types of diabetes.

4.3.5 Correlation between central subfield thickness and chromatic sensitivity

The mean±2σ CST was 282±52.4. A low correlation was found between RG and YB thresholds and the retinal thickness with r^2= 0.15 and r^2= 0.20 respectively (Figure 4-6).

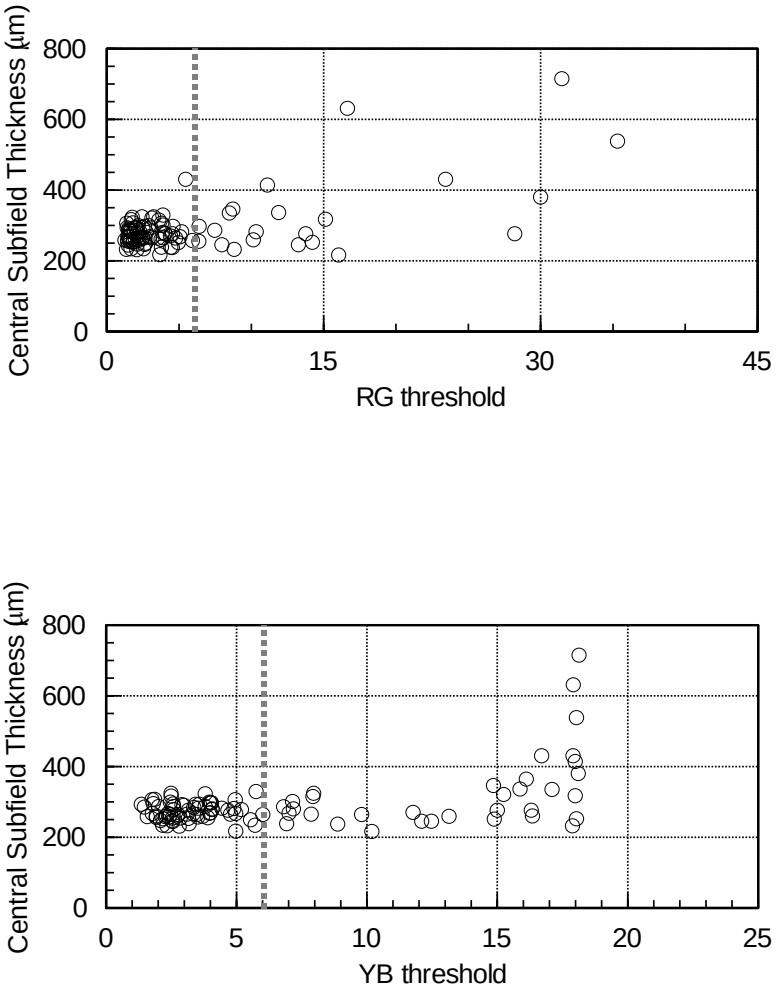


Figure 4-6: Correlation between measured RG and YB colour thresholds and CST as measured in 102 subjects (r^2=0.15 and r^2=0.20 respectively).

4.4 VISUAL ACUITY

In total, 85 subjects (83%) had BCVA 0.00 LogMAR or better (Figure 4-7). This meant that VA was not one of the variables examined in this study.

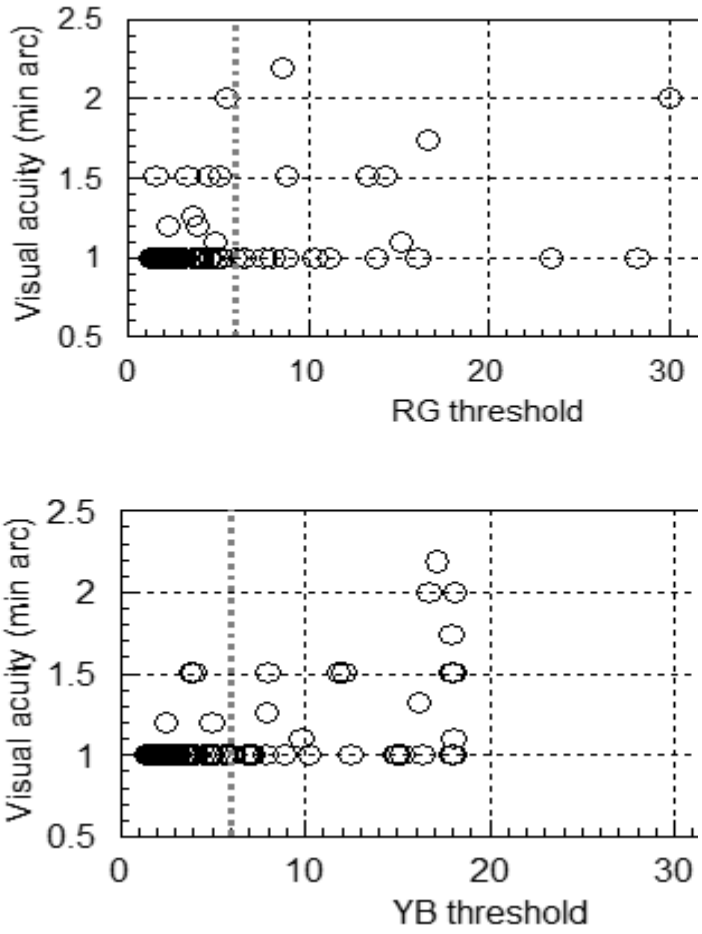


Figure 4-7: VA plotted as a function of RG and YB CAD thresholds.

5 Acuity and colour vision changes post intravitreal Dexamethasone implant injection in patients with Diabetic Macular Oedema

5.1 INTRODUCTION

This chapter describes one of the main complications of DR which is DMO and its most recent modality of treatment which is injection of intravitreal steroid implant that has the ability to reduce the oedema as seen on OCT. We will investigate the pattern of CV changes in such patients prior to and post injection.

DMO affects 20% of patients with DR (Yau et al., 2012) and can cause vision loss independent of the grade of retinopathy.

DMO results from the breakdown of blood retinal barriers and accumulation of extracellular fluid within the inner and outer retinal layers creating diffuse thickening or cystoid spaces that can often extend to affect the whole retinal thickness. Such changes cause displacement in the retinal neuronal components with subsequent temporary or permanent visual loss (Pelosini et al., 2011).

Inflammation is an important factor in the breakdown of the blood–retinal barrier with expression of prostaglandins, leukotrienes and VEGF (Bhagat et al., 2009). As a result, steroids can play a vital role in decreasing intracellular and extracellular oedema through inhibition of these factors by suppression of macrophage activity, vasoconstrictive effect, and reduction of lymphokine production (Abe et al., 1999).

The standard care for DMO has evolved over several years and relies mostly on the control of the systemic condition: diabetes, blood pressure and lipid management. Macular laser photocoagulation was the mainstay of treatment for over 30 years. Direct intervention with focal or grid argon laser photocoagulation to treat areas of retinal thickening led to stabilization of vision (ETDRS report 1, 1985).

More recently anti-VEGF has been introduced for use in the treatment of DMO. In 2012, Ranibizumab became the first medical agent licenced for the treatment of the condition. Findings from two phase III randomised trials (i.e., the RISE and RIDE studies) (Nguyen et al., 2012) show that monthly injections of anti-VEGF Ranibizumab in MO patients lead to a two to three-fold increase in the percentage of patients who gained ≥15 letters in BCVA from baseline at 24 months compared with the sham treatment group.

Steroids can also be useful in the treatment of DMO by blocking the production of VEGF and other inflammatory mediators. Several randomised clinical trials have reported on the use of steroids such as triamcinolone acetonide in the treatment of DMO with improvement in visual acuity and reduction of central retinal thickness (CRT) (Jonas et al., 2003) (DRCR network, 2008) (Gillies et al., 2009). The use of steroids is, however, associated with raised IOP in up to 50% and cataract formation in 40% of the injected eyes (Lowenstein et al., 2006). Similar to ranibizumab the effect is short-lived and patients require frequent injections with higher cumulative risks of side effects adding more constraints on clinical resources.

Sustained release corticosteroids have been developed with the aim of offering longer lasting effects that reduce the need for frequent intravitreal injections. The dexamethasone intravitreal implant - Ozurdex (Allergan Inc., Irvine, CA, USA) is a sustained-release biodegradable implant made of polyacticglycolic acid matrix, containing dexamethasone that is injected into the vitreous through the pars plana using a customized applicator. It releases 700mg dexamethasone slowly into the posterior segment. This treatment demonstrated efficacy in the treatment of chronic DMO and DMO which is resistant to anti-VEGF treatment (Kuppermann et al., 2007; Boyer et al., 2014)

The OCTOME study was conducted in the Laser and Retinal Research Unit of King's College Hospital, London. This was a single centre, exploratory phase III, prospective, open-label clinical study. The study was designed to evaluate the morphological and functional changes following treatment with Ozurdex in patients with MO secondary to DR and vein occlusion. In total, 30 patients with persistent MO were recruited: 24 with DMO, 5 with MO secondary to branch retinal vein occlusion, and 1 with Irvine-Gass syndrome.

OCTOME report 1 confirmed that the therapeutic effect of Ozurdex lasts up to 36 weeks in terms of improvement of visual function and macular thickness in patients with MO. It also concluded that if re-treatment is needed, re-injection of Ozurdex at 20 weeks is appropriate timing in terms of balancing effectiveness with least side-effects (Mathew et al., 2014).

Although measurement of retinal thickness by OCT scan is a useful tool to monitor the response to treatment, it cannot substitute the measurement of visual acuity and both OCT and visual acuity have a place in the monitoring of

diabetic changes. It is however well established that visual acuity does not always correlate well with clinical severity of MO (Browning et al., 2007). Other visual functions such as functional contrast sensitivity, CV and rapid flicker sensitivity may provide a better understanding of the effect of Ozurdex on MO. In this study we measured and compared changes in RG and YB colour thresholds with the corresponding changes in visual acuity and CST to quantify the effects of Ozurdex treatment on other aspects of vision. The patients investigated represent only a small subgroup of the patients recruited for the larger OCTOME study (Mathew et al., 2014).

Our specific aim was to measure and compare changes in RG and YB thresholds using the CAD test (Barbur and Connolly, 2011) (Figures 3-1 and 3-2) in patients with DMO pre- and post-Ozurdex (Dexamethasone implant) intravitreal injection. The study assessed the severity of RG and YB colour loss before treatment and established whether treatment with Ozurdex led to improvement (or at least stabilisation) of chromatic sensitivity. It is also of interest to establish whether changes in chromatic sensitivity can be used to evaluate the efficacy of Ozurdex treatment of DMO.

5.2 METHODS

Subjects were recruited from the Ophthalmology outpatient department of King's College Hospital NHS Foundation Trust, London. The study was approved by the Integrated Research Application System (IRAS) (Ref: 11/NW/0753), King's College Hospital Research and Ethics Committee as well as by the Research and Ethics Committee of City, University of London). The OCTOME study is an interventional, prospective, exploratory study (ISRCTN - 66216819). It was conducted following approval from the Institutional Review Board Ethics Committee (11/H0718/6; NRES committee London Central) and all participants gave written informed consent. The study adhered to the principles of the Declaration of Helsinki.

5.2.1 SUBJECTS

This study examined 14 diabetic patients with DMO. The participants represent a subset recruited from the 24 diabetic patients examined in the OCTOME study. The remaining 10 diabetic patients, not included in this study, had either age related decline in cognitive ability or very poor visual acuity. As a result they were unable to complete the one minute learning stage which does not rely on the use of CV. The ten patients excluded from the study failed to achieve the 100% correct responses required in the learning task which precedes the full CAD test. During the learning task, the CAD stimulus (Figure 3-1 and 3-2) is defined by both colour and luminance contrast and is always seen by both normal trichomats and colour deficients. 100% performance in the learning task

is needed to ensure that the patients understand the requirements of the visual task and can use the keypad response buttons without error.

The OCTOME study had thirty patients in total with MO secondary to different retinal vascular disorders such as DR, branch retinal vein occlusion or following cataract surgery. The exclusion criteria employed in this investigation mirrored the OCTOME study. All participants had to meet the criteria listed below:

- The best corrected visual acuity (BCVA) in the study eye had to be in the range 37 and 68 ETDRS Letters, this level of performance is equal to 1.26 and 0.64 LogMAR acuity, respectively.
- Patients were excluded if they had any other eye disease which could mask or contribute to MO, or any ocular condition in the study eye that would prevent a 15-letter improvement in visual acuity (e.g., severe macular ischemia, extensive macular laser scarring, or atrophy).
- Patients were also excluded if they had advanced glaucoma that was not controlled adequately by drugs alone, or a history of IOP elevation in response to steroid treatment in either eye that resulted in ≥10mmHg increase from baseline with an absolute IOP ≥25mmHg, or required therapy with three or more anti-glaucoma medications.
- Patients were excluded if they had any systemic conditions that precluded trial entry such as known uncontrolled systemic disease or current immunosuppressive disease, initiation of medical therapy for diabetes, or a change from oral hypoglycaemic agents to insulin therapy within 4 months before the screening visit and renal failure requiring haemodialysis or peritoneal dialysis within 6 months before screening visit.

5.2.2 OPHTHALMIC ASSESSMENTS

As part of OCTOME study protocol, all patients were examined every 4 weeks. On every visit patients underwent baseline examination of monocular best corrected visual acuity (BCVA) which was measured using standard ETDRS protocol at 4m distance with a modified ETDRS distance chart. Visual acuity was scored as the total number of ETDRS letters read correctly. This was followed by dilated fundoscopy that was performed using non-contact 90D and digital wide field Volk® lenses, IOP was measured using Goldmann's applanation tonometry, and OCT scans were obtained on Spectralis SD-OCT (Heidelberg engineering GmbH, Heidelberg, Germany). Other visual function tests- monocular as well- were performed at baseline and repeated at week 24. These included contrast sensitivity with the Pelli-Robson chart (Clement Clarke Inc., Harlow, UK) at a distance of 1m and chart luminance of 80–120cd/m^2, RG and YB chromatic sensitivity (measured monocularly in each eye using the CAD test), reading acuity and speed were measured using a standardized protocol with the MNREAD acuity charts (Precision vision, IL, USA), and microperimetry using the Nidek Microperimeter (Nidek Technologies, Padova, Italy). Macular stereo, four field fundus colour photographs and fundus fluorescein angiography were also performed at baseline and week 24.

All patients were refracted by a certified examiner and BCVA for each eye was measured using standard ETDRS protocol at 4m distance with a modified ETDRS distance chart. Visual acuity was scored as the total number of ETDRS letters read correctly. My role included taking medical and ophthalmic history, examining subjects using a slit lamp for biomicroscope, dilated fundoscopy and

IOP measurement, consenting and monocular measurements of chromatic sensitivity, using the CAD test.

All patients had the Ozurdex (Dexamethasone implant) intravitreal injection on week 1. According to the protocol some of the patients were eligible for a second injection at the re-treatment window between week 16 and week 24.

The patient's CV thresholds are compared to the corresponding visual acuity, the measured change in retinal central sub-field thickness (CST) and are presented in this thesis. Other visual parameters measured such as contrast sensitivity, reading speed and central fixation which relate to the OCTOME study have been published under the title OCTOME report 1 (Mathew et al., 2014).

5.2.3 STATISTICAL ANALYSIS

The data were analysed using MS Excel (V.15.0) and SPSS programs (SPSS V.22.0, SPSS, Chicago, IL, USA). Analysis was performed using t-tests to calculate p values and categorical variables were examined using Pearson's r correlation. Differences in results were assumed significant for p-values <0.05.

5.3 Results

5.3.1 Descriptive data

The study included 14 patients. The patients demographic and baseline characteristics are shown in table 5-1.

Mean age (SD), years	56±9.23
Range, years	40-68
Gender, *n (%)*	
Male, *n* (%)	12 (85.7)
Female, *n* (%)	2 (14.2)
Race, *n (%)*	
Afro-Caribbean	4 (28.6)
Asian	2 (14.3)
Caucasian	8 (57.1)
Grade of diabetic retinopathy*, n (%)*	
Mild and moderate NPDR	6 (42.8)
Severe NPDR	3 (21.4)
Treated PDR	5 (35.7)
Smokers*, n* (%)	2 (14.3)
Mean HbA1C*, n* (SD)*	7.8±1.39
≤8%, *n* (%)	6 (50)
>8%, *n* (%)	6 (50)
Hypertensives on treatment, *n* (%)	9 (64.3)
Previous glaucoma medications	0
Previous macular laser, *n* (%)	12 (85.7)
Mean number of laser treatments, *n* (SD)	2.2±1.6
Mean ETDRS letter score*, n (SD)*	59.71±9.20
<54 letters (0.92 LogMAR), *n* (%)	4 (28.6)
≥54 letters (0.92 LogMAR), *n* (%)	10 (71.4)

Abbreviations: ETDRS, early treatment diabetic retinopathy study; HbA1C, glycosylated haemoglobin; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

**n* =12, as data is missing for 2 patients

Table 5-1: Patients demographic and baseline characteristics.

5.3.2 Lens opacity

It has been reported that the age related changes occurring in the human optical system can induce a tritan-like CV defect (Verriest, 1963). The lens yellowing and senile pupil miosis have been attributed to the age related deterioration of CV with a reduction in retinal illuminance (Winn et al., 1994; Verriest, 1963). The density of cataract in the current study was graded using Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993). 12 (85%) of patients were phakic with nine of them graded as N1C1P1, the other 3 were graded as N1C2P1 and the remaining 2 patients were pseudophakic (subjects 6 and 12).

5.3.3 Change in RG and YB thresholds

The mean age in our patients was 56 years (range, 40 to 68) and the age specific, monocular, upper normal limits for a 56 years old subject (i.e., $\mu + 2\sigma$) are 2.66 and 2.85 CAD SNU for RG and YB, respectively. In this study, all the patients were outside the normal age-matched upper threshold limits with the mean RG pre-injection threshold of 22.57 ±11.27 SNU (range, 2.95 to 35.87) while the YB mean threshold was 16.21 ± 3.76 SNU (range, 15.18 to 18.19).

There was significant improvement in RG thresholds post injection (i.e., $\mu + 2\sigma$) 19.18 ±10.84 SNU ($t(13) = 1.965$, $p<0.05$). The post injection YB threshold, although smaller, failed to reach statistical significance ($\mu \pm 2\sigma$: 15.84 ± 4.60 SNU ($t(13) = 0.747$, $p=0.23$). Only four patients showed improvement in their YB threshold (Table 5-2).

The percentage changes in RG and YB thresholds after receiving Ozurdex intravitreal injection are shown in figure 5-1.

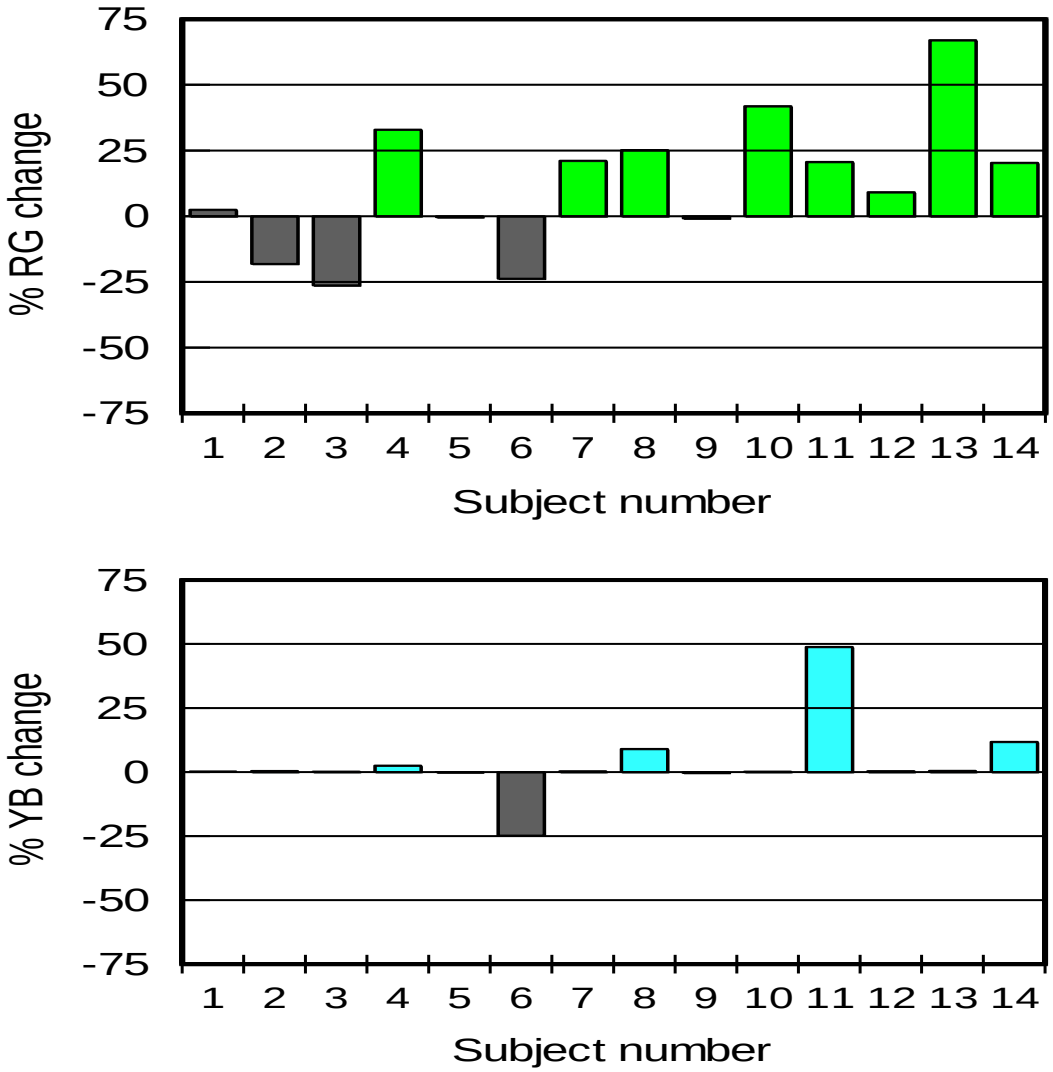


Figure 5-1: Graphs showing the effect of Ozurdex on measured changes in Red/Green (RG) and Yellow/Blue thresholds (YB). 8 subjects showed significant improvement in RG thresholds post injection (between 10-70% improvement). YB thresholds showed less significant improvement, with only 4 subjects showing post injection improvement. All the subjects that showed improvement in RG, also showed improvement YB thresholds.

5.3.4 Change in visual acuity

The number of patients who gained more than 5 or 15 letters by week 24 were 6 (42.8%) and 4 (28.5%) respectively. Three patients (21.42%) lost 2 letters or less, with another patient losing 9 letters by week 24 (Table 5-2).

There was a strong correlation between the percentage improvement of RG threshold and visual acuity ($r(14) = 0.69$; $p<0.05$), while the YB did not show correlation with visual acuity ($r=(14) = 0.04$; $p<0.05$).

The percentage change in VA after receiving Ozurdex intravitreal injection are shown in figure 5-2.

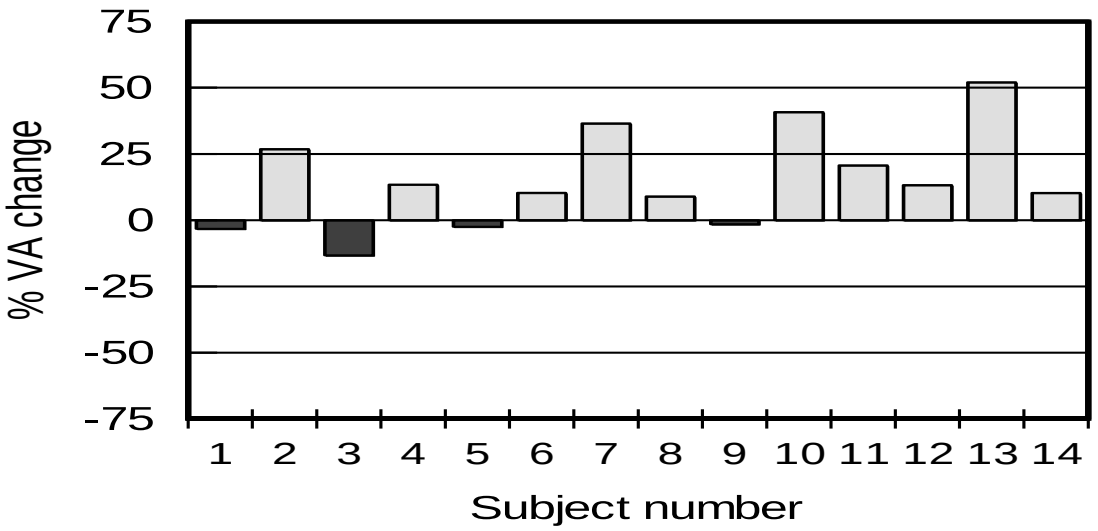


Figure 5-2: Graph showing the effect of Ozurdex on measured changes in visual acuity (VA). 10 subjects (71%) showed 5 or more letters improvement in visual acuity post injection. 4 subjects (29%) experienced worsening of vision by 2-9 letters.

5.3.5 Change in central sub-field thickness (CST)

The $\mu \pm 2\sigma$ CST pre-treatment with Ozurdex was 542 ±135 µm (range, 315 to 881 µm). After treatment and by week 24 the mean$\pm\sigma$ CST has been reduced to 435 ±127 µm (range, 235 to 692 µm) ($t(13) = 2.202$, $p<0.05$).

There was strong correlation between the percentage improvement of RG and CST ($r(14) = 0.78$; $P<0.05$), while the YB threshold changes failed to show significant correlation with CST ($r=(14) = -0.31$).

The percentage change in CST after receiving Ozurdex intravitreal injection are shown in figure 5-3.

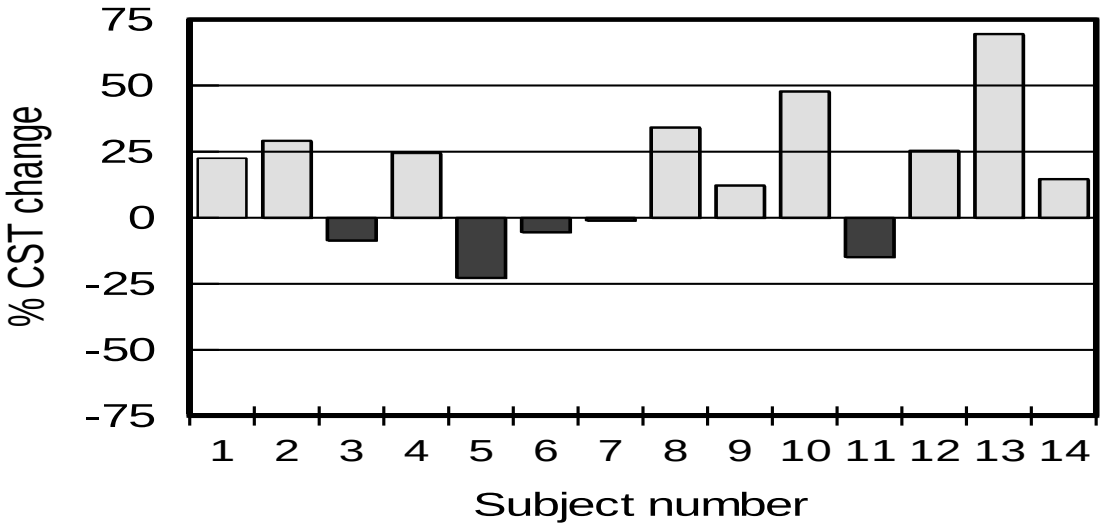


Figure 5-3: Graph showing the effect of Ozurdex on measured changes in central sub-field thickness (CST). 6 subjects showed 25% or more reduction in CST. 3 subjects showed less than 25% reduction in CST while there was increase in CST in 5 patients.

The mean decrease in CST showed an initial significant improvement that was more evident by week 8, then started to show minimal change from week 16 (Figure 5-4).

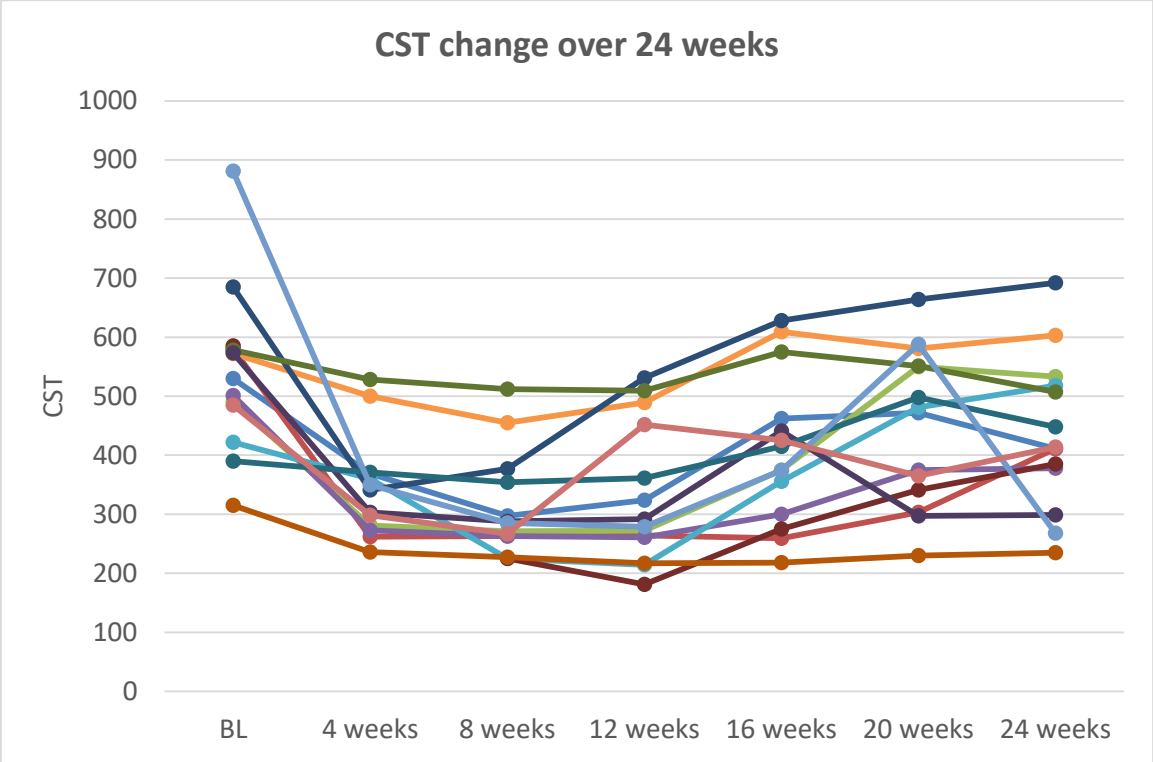


Figure 5-4: Graph showing the change in central sub-field thickness (CST) from baseline over the period of 24 weeks. Significant improvement was more evident by week 8, then started to show minimal change from week 16

Subject	SNO R-G			SNO Y-B			BCVA ETDRS Letters			CST		
	Before	After	*Change*	Before	After	*Change*	Before	After	*Change*	Before	After	*Change*
(1)	31.07	30.30	-0.77	18.15	18.10	-0.05	61	59	-2	530	411	-119
(2)	19.46	22.99	3.53	18.16	18.08	-0.08	67	85	18	580	411	-169
(3)	12.61	15.93	3.32	18.10	18.07	-0.03	68	59	-9	491	533	42
(4)	25.64	17.20	-8.44	18.17	17.71	-0.46	67	76	9	501	378	-123
(5)	34.45	34.58	0.13	18.18	18.18	0.00	40	39	-1	422	518	96
(6)	7.78	9.63	1.85	13.58	16.95	3.37	58	64	6	572	603	31
(7)	28.58	22.54	-6.04	18.19	18.13	-0.06	52	71	19	685	692	7
(8)	11.96	8.95	-3.01	15.70	14.28	-1.42	67	73	6	585	385	-200
(9)	35.87	36.18	0.31	18.10	18.16	0.06	65	64	-1	578	507	-71
(10)	30.51	17.72	-12.79	18.18	18.15	-0.03	49	69	20	573	299	-274
(11)	11.14	8.84	-2.30	11.72	5.99	-5.73	58	70	12	390	448	58
(12)	35.02	31.82	-3.20	18.12	18.05	-0.07	68	77	9	315	235	-80
(13)	29.03	9.57	-19.46	17.52	17.43	-0.09	48	73	25	881	268	-613
(14)	2.95	2.35	-0.60	5.18	4.57	-0.61	68	75	7	485	414	-71

Table 5-2: Change in RG and YB thresholds, visual acuity and CST

5.3.6 Change in visual functions

Other visual functions measured at baseline and at week 24 included contrast sensitivity, reading speed and central retinal sensitivity at 4°. At baseline the contrast sensitivity $\mu + 2\sigma$ was 31.15 ± 3.05, while reading speed was 164.14 ±50.49 wpm and central retinal sensitivity $\mu + 2\sigma$ was 5.63 ± 5.03.

On measuring these parameters again at week 24 it was noticed that there was improvement in all of them, more noticeable though in the retinal sensitivity and reading speed (Table 5-3).

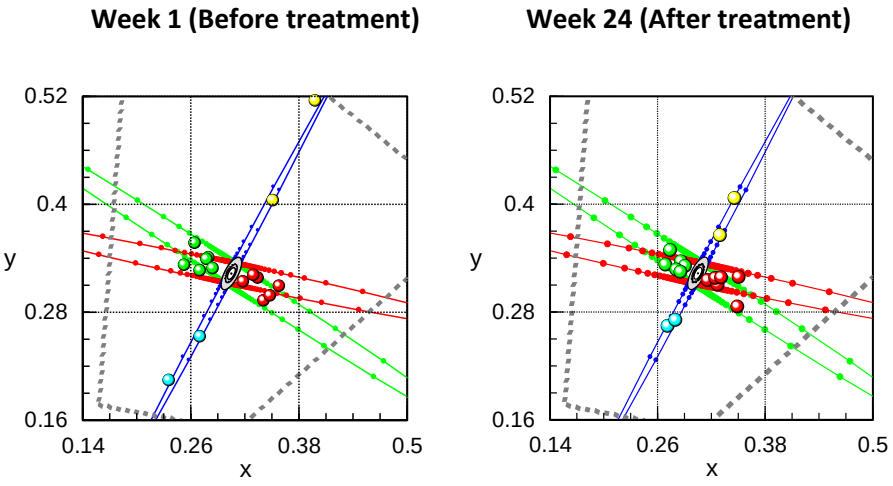
We examined the correlation between the RG and YB thresholds and other visual functions before and after injection as it was of interest to note the high correlation between the contrast sensitivity and the RG thresholds, before: ($r = (14) = 0.60$; $p<0.05$) and after: ($r = (14) = 0.68$; $p<0.05$), respectively. The same high correlation was also present between the RG thresholds and retinal sensitivity, before: ($r = (14) = 0.77$; $p<0.05$) and after: ($r = (14) = 0.73$; $p<0.05$) treatment.

As for the YB thresholds, there was a high correlation with contrast sensitivity before but not after injection, ($r = (14) = 0.64$; $p<0.05$) ($r = (14) = 0.38$; $p<0.05$), respectively. The same was seen with retinal sensitivity with high correlation before but not after injection, ($r = (14) = 0.83$; $p<0.05$) and ($r = (14) = 0.17$; $p<0.05$), respectively.

Visual function	*Baseline*	*Week 24*	*Mean change ± SD*
Contrast sensitivity	31.15 ± 3.05	33.14 ± 2.44	1.99 ± 0.61
Retinal sensitivity 4°	5.63 ± 5.03	10.46 ± 5.86	4.83 ± 0.83
MNREAD reading speed	164.14 ± 50.49	194.14 ± 66.89	30 ± 16.4
Central fixation, *n (%)*	7 (50)	7 (50)	

Table 5-3: Visual functions at baseline and week 24.

Subject A			
Week 1 (Pre-treatment)		Week 24 (Post-treatment)	
RG (SNU)	11.14	RG (SNU)	8.84
YB (SNU)	11.71	YB (SNU)	5.98



Subject B			
Week 1 (Pre-treatment)		Week 24 (Post-treatment)	
RG (SNU)	25.64	RG (SNU)	17.19
YB (SNU)	18.17	YB (SNU)	17.71

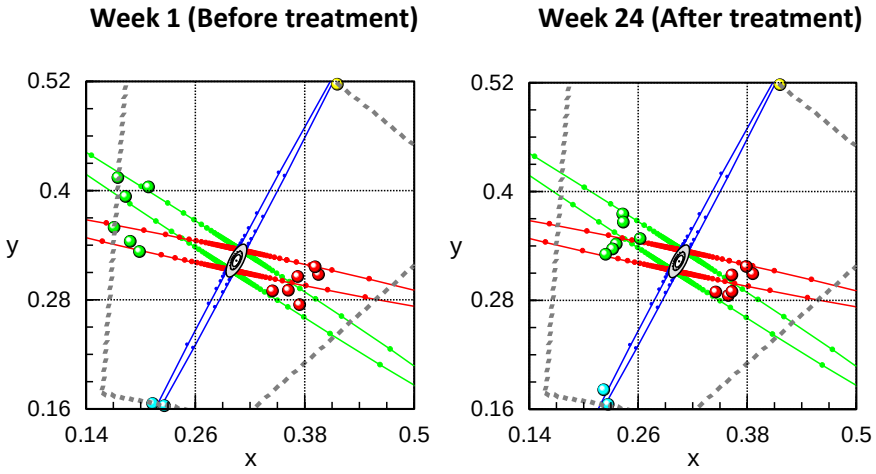


Figure 5-5: The graphs show CAD results before and after Ozurdex treatment in two subjects with diabetes (A=11, B=4). Both subjects show significant improvement in colour sensitivity (i.e., smaller thresholds) post treatment. The grey, dotted lines show the colour limits imposed by the phosphors of the display. Subject B was unable to detect YB colour changes, even for the largest chromatic stimuli that are limited only by the phosphors of the display. Post treatment the YB thresholds remain at these limits with only a small indication of improvement. The RG thresholds, on the other hand, show significant improvement post-treatment.

6 DISCUSSION

The main purpose of this study was to investigate changes in CV in patients with diabetes. In addition, we wanted to ascertain whether it was possible to detect early functional changes in the diabetic retina before structural changes could be detected on clinical examination or fundus imaging. We also wanted to grade the severity of CV loss in comparison to other factors that are normally linked to diabetes, such as the type, grade, control and duration. We also measured changes in CV in a group of diabetic patients with DMO undergoing intravitreal injection of dexamethasone implant (Ozurdex).

The study used the CAD test which has the advantage of being highly sensitive with the ability to quantify the severity of RG and YB loss. The availability of statistical, upper limits for normal CV as a function of age makes the CAD test particularly suitable in detecting and quantifying acquired loss of CV in retinal disease.

To maximise the loss of CV that can be attributed to diabetes, a carefully designed selection process was applied. Those with congenital CV deficiencies, subjects who exhibit RG losses whilst maintaining normal YB thresholds, were not included in the analysis and subjects with any co-pathology that might impair CV were also excluded. Subjects on medications that are known to affect CV were also excluded. So none of the subjects included in this study were taking Digoxin ethambutol, chloroquine, hydroxychloroquine, phenytoin or sildenafil.

The results show that subjects with no DR will exhibit a degree of CV loss which confirms findings suggested in previous study by Feitosa-Santana et al., (2006), who reported on 32 diabetic subjects with no DR. Subjects were tested using the Farnsworth D-15 and the Lanthony D-15d arrangements tests. Results were

compared to 20 age matched normal controls. Their subjects showed compressed colour space configuration along the YB and RG axes in diabetic subjects with no retinopathy. They proposed that these losses resulted from reduced photoreceptor sensitivity rather than being a manifestation of lens yellowing.

To our knowledge, the CAD test has only previously been used to measure binocular thresholds in diabetes without retinopathy (Barbur et al., 2012) and in another small study which was conducted by O'Neil-Biba et al., (2010) that also involved binocular measurements; they measured CV in 7 diabetic patients, 2 with type I and 5 with type II diabetes. Their results demonstrated reduced chromatic sensitivity with varying degrees along the RG and YB spectrum in patients with no or mild DR and no correlation with duration of the disease.

This confirms the influence of the neuronal retinal changes that precedes microvascular changes as reported by Antonetti et al., (2006). These sub clinical changes cannot be detected by conventional methods currently used in diabetic screening such as fundus photography or OCT.

We also found that the severity of CV loss correlates with the severity of DR although this wasn't significant but this is in agreement with a previous study by Roy et. al., (1984), that found correlation between the FM-100 error score and degree of retinopathy. Also in 1998, Ismail and Whitaker reported that the level of mean error score increased with the severity of DR.

The losses in CV thresholds could not be attributed to other diabetes related factors such as duration, type or control. This was similar to findings from previous studies, in 1986, Roy et. al., reported no correlation between the FM-100 error score and fasting blood sugar. A separate study by Kurtenbach et al., (1999), which analysed blood glucose levels in 10 diabetic patients with no signs

of retinopathy, found no correlation between blood glucose levels and the measured CV deficits. Another study by Ong et. al., (2003) failed to show a correlation between CV and duration of diabetes. While other studies did report correlation with duration of disease such as Kurtenbach et al., (1999) and Utkah and Almaca (1992) who reported correlation between the duration and error scores measured in 87 diabetic patients using FM-100 hue test.

Although several studies have found reductions in CV that do not appear to relate to the type, duration or control of diabetes. Unfortunately none of these studies provide a definite explanation as to the exact aetiology or mechanism by which colour vision is affected. The exact processes involved are not understood, but theories have been advanced to account for the observed changes in colour vision. The most common theories include inflammation, apoptosis and glutamate excitotoxicity (discussed in detail in section 2.3.1).

In 2015, Barbur et al. reported on the normal limits for age. These limits were calculated from data obtained from measuring RG and YB thresholds in normal subjects using the CAD test. Similar to our inclusion criteria, any subjects with nuclear, cortical or subcapsular lens opacities higher than grade 2 were excluded from the study. They also excluded any subject with congenital colour deficiency, subjects with medical conditions such as diabetes and hypertension and, or any other subjects that might have any other ocular abnormalities that might cause acquired loss of chromatic sensitivity.

Using this large data set it was possible to calculate the mean thresholds and the corresponding ±2.5σ limits as a function of age. The results showed that above 20 years of age the mean linear increase in RG and YB thresholds is 1% and 1.6% per year respectively.

It was important to establish that the colour threshold changes measured in the subjects investigated in this study reflect the effects of diabetes and not the effects of normal aging of the eye. The results were therefore compared to the upper, normal thresholds for age matched subjects.

In 20 of our subjects, either the RG or YB thresholds (18 for RG and 4 for YB) were within normal limits for the corresponding age. Only two of these subjects had both the RG and YB thresholds within normal limits. As described in the results chapter, the results were analysed both with and without including those subjects but this made no difference to the final outcome.

Chromatic sensitivity has been found to decline with increasing age. This has been attributed to the loss of retinal ganglion cell axons and cell bodies in the neuronal retina (Jonas et al, 1990). Changes to the visual system also take place involving the loss of myelinated axons and alterations to the spatial distribution of myelin in the nerve fibres in the deeper layers of the visual cortex affecting visual processing (Peters at al. 2000, 2001). Colour sensitivity does not appear to be affected by other age related factors such as moderate increases in crystalline lens opacification, changes to the macular pigments and a reduction in the size of the pupil (Werner et al., 2004; Rodriguez-Carmona et al., 2006; Barbur, 2012), provided adequate lighting levels are used during the assessment. In general, these factors cause increased preferential absorption of short wavelength light and this can affect chromatic sensitivity when the ambient light level is low. The effect is not however specific to short wavelength light and can also be demonstrated by using a neutral density filter that causes an overall reduction in retinal illuminance. This may account for the more rapid loss of YB chromatic sensitivity. Stimulus size is also an important variable causing significant loss of both RG and YB chromatic sensitivity (Barbur &

Rodriguez-Carmona, 2012). The CAD test employs photopic levels of retinal illuminance and a large stimulus size to ensure that small changes in retinal illuminance or stimulus size do not affect the measured colour thresholds.

In the other group of diabetic patients with DMO we examined the changes in CV in eyes undergoing intravitreal injection of dexamethasone implant (Ozurdex). All patients showed loss of RG and YB thresholds at recruitment to the study but they responded differently post injection with the majority showing greater improvement in RG sensitivity. This improvement correlated well with the increase in visual acuity. In the related study-OCTOME, the maximum gain in visual acuity was achieved 12 weeks post Ozurdex injection. At 20 weeks post treatment, while the gain in visual acuity started to regress, CV maintained the thresholds that were achieved post injection especially along the RG axis.

The mean decrease in CST showed a similar trend with initial significant improvement that was more evident by week 8, then started to show minimal change from week 16 (Figure 5-4).

Again the mechanisms by which CV is affected in DMO remain poorly understood. Some explanations have been put forward to account for the observed losses, but these have not been satisfactory. Several studies (Barber et al., 1998 & 2011; Nishikawa et al., 2000; Hammes et al., 2003) examined the events of vascular and neuronal apoptosis that occur in DR as the prime cause of CV loss. These studies above focused on the various mechanisms that are thought to cause retinal cell apoptosis (vascular and neuronal retina). Exposure to oxidative stress and reduced growth factor signalling have been identified as

potential factors that may play a key role. Oxidative stress can be induced by hyperglycaemia leading to apoptotic signalling. Growth factor signalling is essential for the survival of neurones, pericytes and endothelial cells. They explained that diabetes impairs the trophic signals pathway leading to reduction in survival signals and hence increasing the chances of apoptosis.

Barber at al., (2003) also demonstrated that glutamate excitotoxicity can cause damage to the neuronal retina and therefore contribute to both chronic and acute neurodegeneration. Neuro-inflammation is also an important factor to be considered in the pathology of DR and subsequent CV defects. Some studies (Seigel et al., 2000; Barber at al., 2001; Gariano et al., 2005) have identified increased levels of cytokines, specially vascular endothelial growth factor (VEGF), interleukin (IL)-1 B, IL-6, IL-8 and tumour necrosis factor (TNF)-α in the vitreous of DMO patients and patients with PDR.

The effect of circulating oxygen saturation on colour thresholds has also been investigated (Dean et al., 1997). The authors examined 37 Type I diabetics with either mild or absent DR. An improvement in colour thresholds was demonstrated in all subjects after breathing oxygen (100%). They concluded that reduced oxygen saturation can lead to impaired CV which is likely to be because photoreceptors are functioning at suboptimal oxygen levels. A similar study conducted by Connolly et al., (2008) showed that chromatic sensitivity was impaired in aircrew when subjected to mild hypoxia as opposed to those under normoxic or hyperoxic conditions.

A majority of our patients showed significant improvement in the morphology of the macula on OCT scan at one month following their injection. This initial

improvement in visual acuity and CST regressed slightly in the following weeks while the initial improvement in chromatic sensitivity was maintained for a longer period, until week 24.

The almost immediate fluid drying effect of the Ozurdex on the macula with restoration of anatomical structure can be seen in OCT images. This does not, however, reflect the extent of damage sustained in the neural retina and can affect some aspects of visual performance. In order to assess the full extent of damage caused by diabetes, the measurement of other visual attributes becomes of interest. Assessment of chromatic sensitivity changes as shown in this study can have an important role in assessing the progress of diabetes and the effectiveness of treatment.

DMO is the major contributing factor to loss of sight in DR. It is therefore important to detect and monitor changes in foveal vision during the course of diabetes. In addition, it is equally important to be able to detect and monitor how visual performance changes during the course of treatment so as to be able to assess objectively the effectiveness of treatment.

All patients who underwent treatment with dexamethasone implant (Ozurdex) had chronic DMO, therefore they would have had previous treatments such as laser - focal or grid, intravitreal triamcinolone and anti-VEGF injections. Given the advanced level of damage to the retina, it was not unexpected to find significant loss of CV in all patients with the YB thresholds being most affected. All of our patients except for two had a degree of cortical and posterior subcapsular cataract which is a known association with diabetes (Olafsdottir el al., 2011). These observations may explain the improvement of RG thresholds, but not in YB thresholds post treatment with Ozurdex.

The findings suggest that RG threshold as quantified with the CAD test can be used to monitor treatment of DMO. At base line all our patients showed higher loss of RG than YB thresholds, but post injection with Ozurdex nine out of 14 patients showed significant improvement in RG thresholds while YB did not show that level of improvement.

The current findings are consistent with the outcome of the OCTOME study in relation to the overall improvement in visual functions post Ozurdex injection. The findings from the OCTOME study "indicate a positive effect on the retinal neuronal function, probably due to a realignment of neuronal structures induced by the drying effect of Ozurdex" (Mathew et al., 2014). The significant improvement in RG chromatic sensitivity observed in this study supports this claim and is consistent with the view that 'A direct neurotrophic effect of Ozurdex cannot be ruled out'.

To our knowledge this is the first study to examine the effect of Ozurdex intravitreal injection on CV. It may be of great benefit in the future to conduct a similar study to investigate changes in CV, contrast sensitivity and rapid flicker in naïve patients with DMO who are receiving Ozurdex as their first line of treatment. Such a study may reveal the full functional benefits of Ozurdex treatment and not just the improvement in BCVA and macular morphology.

CONCLUSIONS

The findings from our study reveal positive correlation between the chromatic sensitivity loss and the severity of DR. It also shows that CV loss occurs in diabetics before any vascular changes are detected by the conventional means currently used in assessing DR such as fundus imaging and clinical examination. These findings suggest that using the CAD test can provide a sensitive means of measuring colour thresholds to reflect the health of the retina and detect any functional changes that precede structural changes which is important in monitoring the progression of DR.

Also our study of DMO patients reveal significant improvement in RG colour thresholds as a result of treatment with Ozurdex, with little or no improvement in YB thresholds. The improvement in chromatic sensitivity correlates well with the associated improvement in visual acuity and CST. In addition to VA and OCT measurements, the findings from this study suggest that changes in CV and particularly in the RG chromatic mechanisms can provide a useful biomarker in monitoring the efficacy of treatment in DMO.

LIMITATIONS AND FUTURE WORK

The main limitation to our study was the difference in distribution of numbers of subjects included in each of the group. We had less number of subjects recruited in group 2 (moderate or severe) and group 3 (treated PDR) and this is attributed to the efficacy of current screening programmes as we are seeing less and less numbers of patients with advanced DR in the clinics.

It would be of great interest to follow-up on the patients who had no signs of DR but still exhibited a degree of CV loss to establish whether they develop clinical signs later. Also the other groups of patients to detect if a change in the grade of DR will correlate with change in their CAD test thresholds. A longitudinal study in the future would be very useful to establish this.

The findings from the study of patients treated with Ozurdex for DMO would have been more convincing with a larger sample size. Unfortunately not all of the diabetic subjects recruited in the OCTOME study were able to perform the CAD test, either due to limited cognitive ability or because of severe loss of vision which hindered their ability to complete successfully the one minute learning stage of the test. The CAD test does offer the option of increasing the stimulus size which may have allowed some of these patients to carry out the test. This may have, however, been of limited value as we would not have been able to correlate it with the remaining parameters that would have already been affected by the extensive loss of vision. It would be useful to design a study to include treatment naive DMO patients rather than chronic DMO patients. The patients that we were able to examine exhibited greater loss of YB sensitivity

compared to RG which may have contributed to the fact that lens opacity can reduce retinal illuminance levels and that this in turn can cause a significant reduction in YB sensitivity. This may explain why post injection there was much greater improvement in measured RG thresholds as compared to YB thresholds. As per the protocol of the OCTOME study the CAD test was only done once after the injection, at week 24, but it may be useful in future studies to examine the patients again beyond week 24 to find out if the observed improvement in RG colour thresholds is sustained.

APPENDIX A. PATIENT INFORMATION SHEET

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Changes in visual function as predictors of Ocular diseases

Patient Information Sheet:

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

Professor Barbur of City University and Miss Sivaprasad of King's College Hospital and their team are currently investigating the possibility of early detection of potential retinal changes in ocular diseases.

Why have I been invited?

You have been invited to consider taking part in this research study because you have been diagnosed with ocular disease (AMD, Diabetes or Glaucoma).

Do I have to take part?

It is up to you to decide whether or not to take part. We will describe the study and you will be given this information sheet to keep. If you do decide to take part, we will then ask you to sign a consent form to show you have agreed to take part. Participation in the study is voluntary and will not affect your current or future medical care or management. You may withdraw from the study at any stage, without giving a reason, without your legal rights being affected.

What will happen to me if I agree to take part?

Member of the research team will explain to you all the procedure that will be involved when taking part in the study. You will be asked to sign a consent form and you will be given a copy of this information sheet and the consent form to keep.

Version 1 18.08.2011

Your treatment plan will be exactly the same, whether or not you decide to take part in the research study.

What will I have to do?

For the study we will carry out colour vision test that is designed to assess small changes in colour vision and detection of flicker. The test requires you to make judgements based on what you see on a visual display (much like a TV screen). All techniques are non-invasive (there is no contact with the eyes) and it will not result in any discomfort or blurring of your vision. No eye drops will be used during the test. If you wear spectacles or contact lenses, you will need to wear them during the test.

What is the procedure that is being tested?

The purpose of the study is to validate a simple test developed at City University which can detect early changes in the eye caused by certain ocular diseases.

What are the alternatives for diagnosis?

The current alternative process for diagnosis is carried out using standard clinical tests which detect the condition based on changes noticeable by the patient at a much later stage than proposed by our new tests.

What are the possible disadvantages and risks of taking part?

Your treatment plan will be exactly the same whether or not you decide to take part in the research study.
There are no risks of involvement in the research study. Participation in the study will not affect your legal rights.

What are the possible benefits of taking part?

There will be no direct benefit to you from participating in this study, but the information we get from this study we hope will help improve the treatment of people with AMD, diabetes and Glaucoma.

What if there is a problem?

Complaints
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed.

Version 1 18.08.2011

APPENDIX B PATIENT CONSENT FORM

CITY UNIVERSITY LONDON

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CONSENT FORM

Title of project: Changes in visual function as predictors of ocular diseases

	Please initial box
1. I confirm that I understand the nature and demands of the research explained to me and I agree to participate in the project. I also confirm that I have read and understand the information sheet dated 18.08.2011 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	☐
3. I understand that any information I provide is confidential, and that no information that could lead to the identification of any individual will be disclosed in any reports on the project, or to any party outside the project.	☐
4. I understand that this is a research investigation and that the results cannot be used for diagnosis.	☐
5. I understand that relevant sections of my medical notes may be looked at by responsible individuals from Kings College Hospital where it is relevant to my taking part in this research study. I give permission for these individuals to have access to my records.	☐
6. I agree to my GP being informed of my participation in the study	☐
7. I agree to take part in the above study.	☐

Version 1 18.08.2011

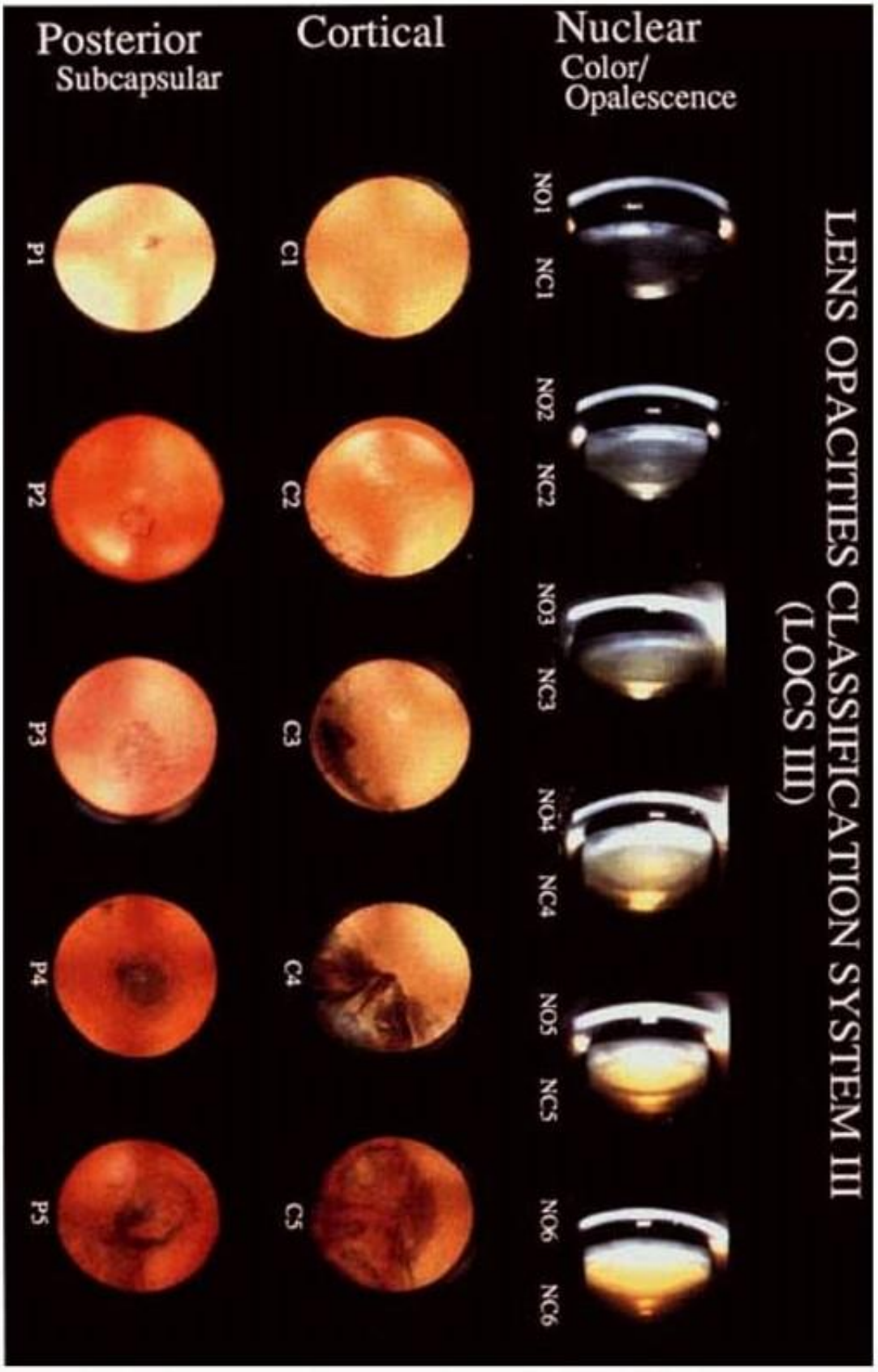
8. **I am / am not** willing to be contacted in the future regarding the possibility of participation in similar research studies (delete as appropriate). ☐

Name of participant	Date	Signature
Name of person taking consent	Date	Signature

When completed: 1 copy for participant; 1 copy for researcher site file

Version 1 18.08.2011

APPENDIX C LENS OPACITIES CLASSIFICATION SYSTEM III (CHYLAK ET AL., 1993)



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