Structure and function of the visual pathway in demyelinating optic neuropathy

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Background

Philosophers defined the eye as a window to the soul long before scientists addressed this cliché to determine its scientific basis and clinical relevance. Anatomically and developmentally, the eye is an extension of the central nervous system (brain). It consists of retinal ganglion cells, the axons of which form the optic nerve, whose fibres are, in effect, CNS axons. The eye has unique physical structures and a local array of surface molecules and cytokines, and is host to specialized immune responses similar to those in the brain and spinal cord. Several well-defined neurodegenerative conditions that affect the brain and spinal cord have manifestations in the eye.

Multiple Sclerosis is a multi-factorial autoimmune disease leading to variable disability in early life. This disabling condition has impact on social and occupational life and eventually affects quality of life and vision. MRI is the main stay for early diagnosis and to determine response to treatment. Early diagnosis and subsequent treatment with disease modifying therapy has shown to have better outcomes on a long-term basis.

The Retinal nerve fiber in the optic nerve and retina is devoid of myelin that makes it an ideal site for study of axonal damage. The loss of axons can be detected by a simple scan called, Optical coherence tomography that uses the principle of interferometry. This makes imaging free of radiation, less time consuming, cost effective and above all, OCT has a high inter-test and intra-test reliability ma

king it both specific and sensitive. OCT with different protocol can assess optic nerve, macula and choroid structurally which can then be correlated with sensitive functional measures of vision.

This project aims to determine the role of structure and assessment of function of the visual pathway in Multiple sclerosis. It explores the role of vision studies in diagnosis of MS and to determine progression of disease.

Further, we explore the correlation between visual outcomes with that of neurological outcome in multiple sclerosis. Sensitive visual testing for functional assessment along with precise technology of optical coherence tomography (scan of retina) for structural assessment has been used. Sub group analysis for the optic neuritis group and disease modifying treatment group has been studied. Intra and inter eye correlation have been derived to make a final conclusion for the role of vision study in Multiple Sclerosis.

Does assessment of structure and function of visual pathway in MS have a future? Does it help in diagnosis, determining prognosis and response to treatment in relapsing remitting Multiple sclerosis?? Does structure meet function!!

Abstract:

Introduction:

Multiple sclerosis is a disabling disease with impact on the social, financial and occupational life of an individual. Diagnosis of Multiple sclerosis even with the McDonald criteria, sometimes can take many years, by which time the patient has already accumulated significant disability. Hence the need for early diagnosis and prompt treatment for a better prognosis highlighted in different MS studies.

Visual dysfunction in patients with Multiple sclerosis is a common finding. Asymptomatic visual loss has been highlighted in various studies. In the United Kingdom, studies have focused on genetics, environmental factors, assessment of neurological state (EDSS, MSFC) along with MRI of the brain and spinal cord. There is no single study in the UK that has looked at the visual pathway in Multiple sclerosis and correlated with the neurological outcomes.

Aim:

Correlation of structure and function of visual pathway in Multiple Sclerosis

Methodology

We recruited 55 (110 eyes) patients with relapsing remitting MS and 25 controls (50 eyes) into this project with specific inclusion and exclusion criteria. Power calculations were done based on the parameters used for the study. Coefficient of reliability and variability was tested prior to the study in a subset of control patients. Logistic regression model with odds ratio was used to assess the ability of each test to differentiate disease from control. Subgroup analysis was done for the optic neuritis group and the group receiving disease-modifying therapy.

Function of the visual pathway was assessed using Sloan contrast charts along with HRR colour chart and HVF indices. Structure of the visual pathway was mainly assessed using different protocols from Optical Coherence Tomography. Ethical approval was obtained through the North Midlands IRAS and Keele University.

Results

The structure of the visual pathway correlates significantly with function of vision. This in turn correlates with neurological function.

Visual tests are more sensitive than neurological testing by EDSS and hence can be used in diagnosis and to determine prognosis in RRMS.

GCIP index, EDI OCT and posterior pole protocols on OCT have revealed useful and valuable information that can be translated into clinical use to manage patients with MS.

Conclusion

Sloan contrast charts and HRR colour testing are sensitive measures of disease progression. Visual field test indices on the Humphrey's field test closely relate to RNFL thickness on posterior pole OCT.

GCIP and EDI-OCT show different clinico-pathological staging in MS.

Visual parameters have synergistic power in evaluating the disease process in MS. Visual testing for structure and function is a valuable addition to the routine assessment of MS patients. Visual assessment helps in detection of the spectrum of disease in MS with specifics to the range of manifestations and severities of illness .Multiple sclerosis like many other diseases exhibit the iceberg phenomenon. The iceberg phenomenon describes a situation in which a large percentage of a problem is subclinical or otherwise hidden from view. Thus, only the "tip of the iceberg" is apparent to the clinician. Visual assessments help in uncovering disease below the sea level by early detection and subsequent better disease control. Visual studies in MS using the parameters in this study must be considered for routine use in our day to day practice in diagnosis and managing patients more effectively.

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AON	Acute optic neuritis
AQ-4	Aquaporin-4
ACTH	Adrenocorticotrophic hormone
ARR	Annualised relapse rate
AVP	Anterior visual pathway
BV	Binocular vision
BY	Blue yellow
BO	Binocular optic neuritis
BSimON	Bilateral simultaneous optic neuritis
BSeqON	Bilateral sequential optic neuritis
BNB	Blood nerve barrier
BRB	Blood retinal barrier
BSCB	Blood spinal cord barrier
BCNSB	Blood central nervous system barrier
BB-EC	Blood brain endothelial cell
BBB	Blood brain barrier
CT	Computed Tomography
CNS	Central nervous system
CSF	Cerebrospinal fluid
CCSVI	Central cerebrospinal venous insufficiency

CIS	Clinically isolate syndrome
CRION	Chronic relapsing inflammatory optic neuritis
COST	Cone outer segment tips
CPSD	Corrected pattern standard deviation
DMT	Disease modifying treatment
Db	Decibels
D	Dioptre
EDI	Enhanced depth imaging
EDSS	Expanded disability scoring system
ETDRS	Early treatment diabetic retinopathy screening
EZ	Ellipsoid zone
ELM	External limiting membrane
EPL	External plexiform layer
EBV	Epstein Barr virus
FDA	Food and drug authority
GCCA	Ganglion cell complex area
GDA	Glaucoma diagnostics
GCIP	Ganglion cell inner plexiform
GCL	Ganglion cell layer
GPRD	General Practice research database
GDX	Glaucoma diagnostic
HLADR2	Human Leucocytic Antigen DR2

HLA	Human Leucocytic Antigen
HRT	Heidelberg Retinal tomogram
HVF	Humphrey's visual field
HRR	Hardy Rand Rittler chart
IQR	Inter quartile range
ITCB	Ishihara test for colour blindness
IZ	Inter-digitation zone
ILM	Internal limiting membrane
IPL	Internal plexiform layer
INL	Inner nuclear layer
IS	Inner segment
ICD	International classification of disease
IV	Intravenous
LGN	Lateral geniculate nucleus
LCVA	Low contrast visual acuity
LogMAR	Logarithmic Minimal angle of resolution
LC	Lamina cribrosa
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MMP	Metalloproteinase
MRV	Magnetic resonance venography
MHC	Major histocompatibility complex

MSON	Multiple sclerosis optic neuritis
MSFC	Multiple sclerosis functional composite
MD	Mean deviation
mm	millimeter
MSQOL	Multiple sclerosis quality of life
MD	Mean deviation
MSFC	Multiple sclerosis functional composite
NON MS	No optic neuritis Multiple sclerosis
NMO	Neuromyelitis optica
IgG	Immunoglobulin G
NICE	National institute of clinical excellence
NFL	Nerve fibre layer
NHS	National Health Service
n/ N	Number
NEI	National eye institute
OD	Right eye
OS	Left eye
ONMS	Optic neuritis Multiple sclerosis
OPA1LW	Opsin gene for L cone
ONTT	Optic neuritis treatment trial
ONH	Optic nerve head
OCT	Optical coherence Tomography

ONL	Outer nuclear layer
OS	Outer segment
OT	Optic tract
OCH	Optic Chiasm
ON	Optic nerve
OSMS	Optico spinal Multiple sclerosis
PPMS	Primary progressive Multiple sclerosis
PMBC	Papillomacular bundle complex
PML	Progressive multifocal leucoencephalopathy
PE	Pigment epithelium
PSD	Pattern standard deviation
P value	Probability value
PPRNFL	Peripapillary retinal nerve fiber layer
QOL	Quality of life
QOV	Quality of vision
RON	Relapsing optic neuritis
RRMS	Relapsing remitting multiple sclerosis
ROS	Reactive oxygen
RIS	Rod inner segment
RGC	Retinal ganglion cells
RNFL	Retinal nerve fibre layer
RG	Red green

SWAP	Short wavelength automated perimetry
SD	Standard deviation
SLCVA	Sloan Low contrast visual acuity
SF	Short term fluctuation
SITA	Swedish interactive threshold algorithm
SD OCT	Spectral domain Ocular coherence tomogram
SLP	Scanning laser polarimetry
SION	Single isolated optic neuritis
SPMS	Secondary progressive Multiple sclerosis
T-MRI	Tesla Magnetic resonance imaging
TV	Total volume
UHNM	University Hospital of North midlands
UK	United Kingdom
VA	Visual acuity
VEP	Visual Electro Physiology
VF	Visual field
VCC	Variable corneal compensation

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Chapter 2

Introduction to Multiple sclerosis (MS)

2.1 Historical: The story of Multiple sclerosis and Vision

The earliest of description of multiple sclerosis (MS), possibly, dates back to the 14th century. A number of documents written before or shortly after the death of a young Dutch woman, Lidwina of Schiedam (1380-1433) [1]surprise today's clinicians by their very accurate description of symptoms which for the most part correspond to the clinical criteria prevailing nowadays for the diagnosis of multiple sclerosis. These could be the oldest known document, describing a case of multiple sclerosis (Medaer, 1979) [2].

The virgin Lidwina of Schiedam (1380–1433) [1] fell while skating at age 16. She had an illness over the next 37 years that had many of the features we would now identify as multiple sclerosis, but the diagnosis must remain as "possible MS." (Medaer) [2]

Pope Leo XIII canonized Saint Lidwina in 1890. She is listed in lives of the Saints, with "saint day," April 14. Online Saints lists her as the patron saint of sickness, and because of her fall while skating, she is also the patron saint of figure skating. The United States Figure Skating Association has a medal featuring a picture of Lidwina.

It was the French neurologist, Jean-Martin Charcot (1825–1893) [3], who made the first definite links between the symptoms of MS and the pathological

changes seen in post-mortem samples. He described the condition as “sclerose en plaques” and recognised MS as a distinct disease entity. Charcot’s contribution extended to the development of diagnostic criteria, which included the now-famous triad of “nystagmus, tremor and scanning speech”. He also identified many important histological features, including loss of myelin (Lublin 2005) [4].

Following Charcot’s landmark description, knowledge of MS became widespread and an entire chapter was dedicated to the condition in Gower’s “Manual of diseases of the Nervous system” (Gower 1893) [5].

In 1845, before the invention of the Ophthalmoscope to visualise the back of the eye (fundus), diseases of the fundus, were impossible to recognise. Ancient physicians divided all eye diseases into “ophthalmia” or “blindness”. “Ophthalmia” included all varieties of conjunctival and corneal diseases recognised through inspection of the globe. “Blindness” referred to vision loss not based on an obvious change in the visible surface or media of the eye. Blindness was often considered a divine punishment for sin. Within that group, optic neuritis may have accounted for some of the “miraculous” spontaneous cures.

The earliest references to optic nerve dysfunction as a mechanism for vision loss are found in Arabic texts of ophthalmology written in the ninth century. In

what is believed by some to be the first major textbook of ophthalmology, HunainIbn Is-Haq[6] described three different forms of paralysis of the eye: those involving perception alone, those involving eye motion alone, and those involving both [6]. In these early descriptions, he does not distinguish optic neuritis from other diseases of the posterior segment. However, there are specific references in this volume to pain and heaviness, "swelling" of the optic nerve, and the afferent pupillary defect [6]

Another Arabic writer, Ali Ibn Isa, referred to inflammation of the optic nerve, although he may have been describing papilledema, as he related "the cause of the blindness to the ventricles of the brain." [7] In his textbook of ophthalmology, Isa describes various affections of the optic nerve, including those resulting from "warmth, cold, humidity etc".

In one of the earliest English language textbooks of ophthalmology, published in 1823, George Frick [8] wrote that optic neuritis, without the advantage of the ophthalmoscope, it was impossible to distinguish uveitis, migraine, retinal detachment, optic nerve and orbital apex disorders.

Frick makes reference to severe pain in the orbit preceding vision loss, abnormal responses of the pupil to light, and the visual behaviour of patients with central scotoma.

Manuals written by Saunders [9] in 1821 and Littell[10] in 1846 present a similarly confused impression of optic nerve and retinal diseases.

Optic neuritis in post-ophthalmolscopic era

With the widespread use of the ophthalmoscope in the second half of the nineteenth century, the various diseases affecting the optic nerve and retina became distinguishable. Von Graefe first described optic neuritis with some uniformity in 1860 [11] and so did Nettleship in 1884 [12]. Nettleship acknowledged that Leber, Hutchinson, and Hock had previously described cases of optic neuritis. Nettleship points out confusion with tobacco amblyopia in Leber's writing and describes Hutchinson's series as having a variable group of patients and Hock as describing the characteristic pain of optic neuritis

In 1930,Brain (journal) [13] wrote an in-depth review of the subject. Brain's review shows a shift in the emphasis of symptomatology with Charcot's triad of nystagmus, intentional tremor and dysarthria only being in minority of cases. The great variety of clinical features was now the important issue with common earliest symptom being parasthesia, motor and visual symptoms. Onset was recognised as usually sudden with relapses and remissions although cases of more insidious onset were becoming increasing recognised.

Brain records a similar description of visual signs and symptoms. Amblyopia was still a common presenting symptom and pallor of the optic disc recorded in

33% to 58% of cases. Direct ophthalmoloscopy, visual acuity and visual field testing were still the main stay of examination of the visual system. The visual field in MS had received much attention and the detection of central scotoma and peripheral constriction has been explored.

Klingman (1910) [14] had described paracentralscotoma, especially in the temporal field and Traquair (1925) [15] described retrobulbar neuritis affecting the optic chiasm and tract. Cerebro-spinal fluid examination has been used since 1920.A section on the investigation of MS in a chapter in Wilson's book "Neurology"(1940) [16] was essentially unchanged from twenty years before.

In the process of finding the cause and renaming the disease, Multiple sclerosis society was formed on the 30th July 1945[17]. The formation of these societies were consolidated in 1955 with publication of the monograph Multiple Sclerosis written by Douglas McAlpine (1890–1981), Nigel Compston (1918–1986) and Charles Lumsden (1913–1974) since when the condition has universally been known as multiple sclerosis.

John Kurtzke (1988) [19] has reviewed the history of the naming of multiple sclerosis and the classifications that were introduced for epidemiological purposes in order to weight the diagnosis in the absence of pathological proof. Allison and Millar (1954) [20] classified cases as early (few physical signs but a recent history of remitting symptoms), probable (soon changed to early

probable or latent: no reasonable doubt about the diagnosis), possible (findings suggesting the diagnosis and no other cause found but the history static or progressive and with insufficient evidence for scattered lesions) and discarded disseminated sclerosis.

Until the mid-1980s, all surveys of multiple sclerosis used the Allison and Millar criteria with some modifications within categories, including introduction of the term (clinically) definite (Bauer et al 1965 [21]; Broman et al 1965 [22]). The principles developed by John Kurtzke in classifying United States army veterans, on which consensus was later reached by a panel of examining neurologists, was formalized by Schumacher et al [23] who categorized definite cases as those showing objective evidence for disease affecting two or more white matter parts of the central nervous system (CNS), occurring in episodes separated by >24 hours or with progression over 6 months, in a person aged between 10 and 50 years at onset and in whom a competent observer can find no better explanation. (Compston et al, McAlpine's Multiple Sclerosis 2006) [24.]

Visual evoked response (VER) have been recognised since the origin of electroencephalography (EEG). Adrian et al [25] demonstrated that regularly repeated flashes of light elicited electrical responses from the surface electrodes over the occipital cortex. Lord Adrian shared a noble prize with Sir Charles Sherrington shared a noble prize in 1932 for their work in Neurophysiology.

The type of reversing chequerboard pattern now commonly used owes it origin to the work of Spekreijse[26]and Cobb et al [27]. These techniques made it possible to identify both the average response to a reversing chequerboard patterns well as the response to the onset and offset patterns. A paper by Halliday et al [28], on delayed evoked potential in optic neuritis was a driving force for laboratories to be set up with evoked potential tests.

Imaging techniques such as Air encephalography, Boudin et al [29]; myelography, Haughton et al[30], were all studied as diagnostic aids but results were poor until the advent of Computed tomography (CT). However, CT was superseded within a few years by Magnetic resonance imaging (MRI) as first reported by Young et al., [31]. Imaging techniques have rapidly developed in the last thirty-five years and is one of the main stay in the diagnosis of MS.

While the investigations to investigate brain and the spinal cord were gaining importance, there was a missing piece of information in this multi-modal, inflammatory, autoimmune condition that affected the central nervous system. The visual pathway, an embryological part of the forebrain was not looked into until recently in the 20th century gaining speed and refinement in the recent times. This opens a whole new concept of approaching the disease through the **EYE**, **is the "window to the brain".**

2.2. Multiple Sclerosis, the disease

Multiple sclerosis (MS) involves an immune mediated process in which an abnormal response of the body's immune system is directed against the central nervous system (CNS), which is made up of the brain, spinal cord and optic nerves/pathway. The exact antigen or target that the immune cells are sensitised to remains unknown, which is why MS is considered by many experts to be "immune-mediated" rather than "autoimmune condition.

Within the CNS, the immune system attacks myelin, the fatty substance that surrounds and insulates the nerve fibers as well as the nerve fibers themselves. The damaged myelin forms scar tissue (sclerosis), which gives the disease its name. When any part of the myelin sheath or nerve fiber is damaged or destroyed, nerve impulses travelling to and from the brain and spinal cord are distorted or interrupted, producing a wide variety of symptoms.[32]

2.3 Epidemiology

An estimated 1.1 to 2.5 million people worldwide suffers from multiple sclerosis [33]. Although the reason is unclear, this condition is more common in regions that are farther away from the equator. In Canada, parts of the northern United States, western and northern Europe, Russia, and south eastern Australia, the condition affects approximately 1 in 2,000 to 2,400 people. It is less common closer to the equator, such as in Asia, sub-Saharan Africa, and parts of

South America, where about 1 in 20,000 people are affected. For unknown reasons, most forms of multiple sclerosis affect women twice as often as men [34]; however, women and men are equally affected by primary progressive MS. The prevalence of MS recorded in GPRD (general practice research database)has increased by about 2.4% per year, reaching 285.8 in women and 113.1 in men per 100 000 by 2010. There was a consistent upward trend in incidence of MS reaching 11.52 in women and 4.84 in men per 100, 000/year by 2010. Peak incidence occurred between ages 40 and 50 years and maximum prevalence between ages 55 and 60 years. Women accounted for 72% of prevalent and 71% of incident cases. Scotland had the highest incidence and prevalence rates in the UK. An estimate that
126,669 people were living with MS in the UK in 2010 (203.4 per 100 000 population) and that 6003 new cases were diagnosed that year (9.64 per 100 000/year). There is an increasing population living longer with MS, which has important implications for resource allocation for MS in the UK[35]

2.4 Etiology

Etiology of multiple sclerosis (MS) is still unknown, but both the genetic and environmental fields contribute to the emergence of the disease. Of the environment, in particular, prospective cohort studies revealed in recent years that prior infection with Epstein-Barr virus (EBV) [35], deficiency of vitamin D

[36], and tobacco smoking [are] important for the generation of MS in a multifactorial way. With diet, as one of the possible causes of MS, the situation is at present inconsistent. In many reviewing articles of its epidemiology, diet is also mentioned as a factor of importance. Other risk factors relate to age, gender and ethnicity. Women are 2 to 3 times more likely to develop MS than men. MS usually strikes between the ages of 20 and 40 with higher risk in patients of northern Europe descent.

2.5 Genetic etiology

Genetic of MS:

The MS genetic consortium and our research group have thrown light into the role of genetics in MS[37, 38.]

Genetics of Optico spinal MS (OSMS)

Multiple Sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) while Neuromyelitisoptica (NMO) selectively affects the optic nerves and spinal cord. In Asians, MS is rare; however, when it appears, the selective and severe involvement of the optic nerves and spinal cord is characteristic. This form, termed optico-spinal MS (OSMS) has similar features to the relapsing form of NMO in Western populations.

Because NMO-IgG have been reported to be present in 30-60% of OSMS patients, OSMS in Asians has been suggested to be the same entity as NMO [39]. The Opticospinal multiple sclerosis showed a significantly higher age of onset, higher expanded disability status scale scores and higher CSF cell counts and protein content than the Western type multiple sclerosis. On brain and spinal cord MRI, the opticospinal multiple sclerosis showed a significantly lower number of brain lesions, but a higher frequency of gadolinium-enhancement of the optic nerve and a higher frequency of spinal cord atrophy than in Western type multiple sclerosis. The frequency of the HLADPB1*0501 allele was found to be significantly greater in opticospinal multiple sclerosis (93%) than in healthy controls (63%, corrected P value = 0.0091 and relative risk = 7.9), but not in Western type multiple sclerosis (66%) or spinal multiple sclerosis (82%)

2.6 **Patho-physiology of MS**

The primary cause of MS is currently unknown. Current models can be divided in two groups, Inside out and Outside-in.

In the inside-out model, it is hypothesised that a problem in the CNS cells produces an immune response that destroys myelin and finally breaks the blood-brain barrier (BBB). In the outside-in models, an external factor produce BBB leaks, enters the CNS, and destroys myelin and axons.

Direct influence of immune cells on the BBB

The BBB has direct influence from the effect of cytokines, ROS and matrix metallo-proteinases (MMPs) produced by peripheral blood mononuclear cells (PBMCs) that can directly disrupt components of the BBB or act on receptors expressed by BBBECs (Fig. 2.1)

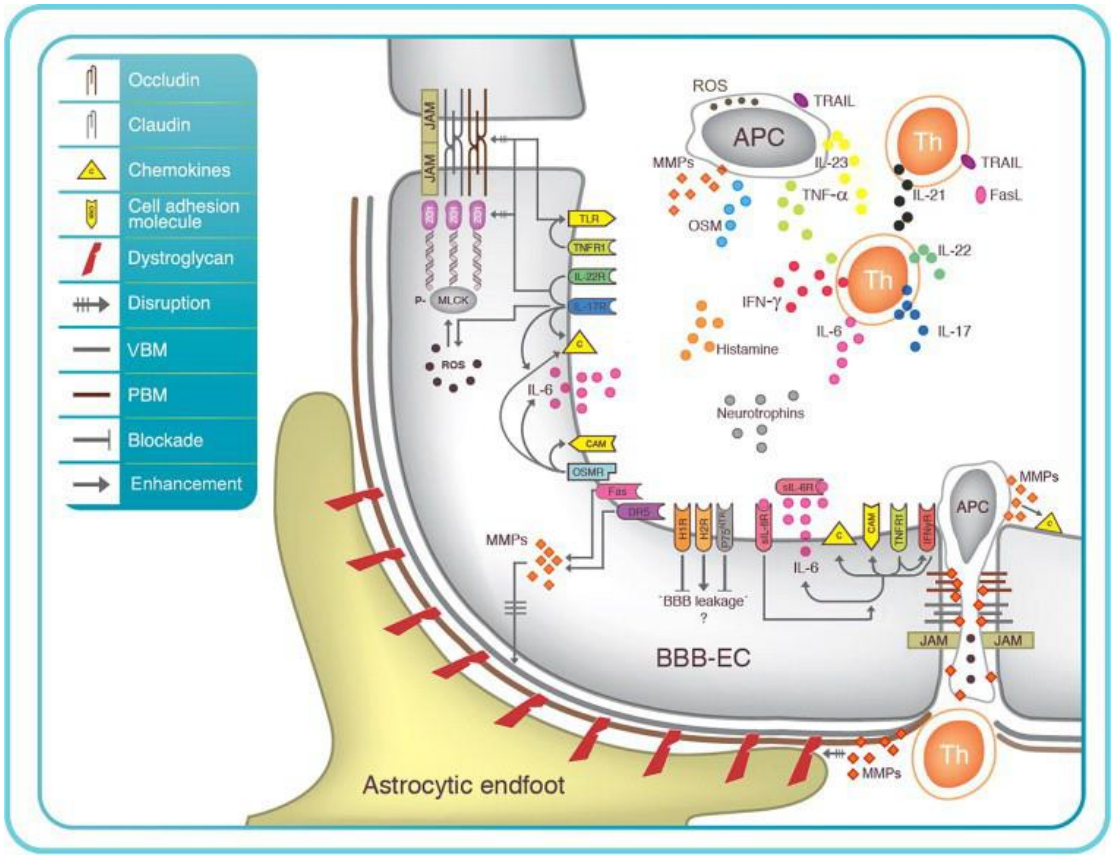


Figure 2.1: Blood brain barrier (Textbook of Neurology (1997). "The Blood-Brain Barrier in Neuroinflammatory Diseases"**)**

2.7 The blood -brain barrier

The central nervous system (CNS), along with testis and eye, is considered an immune privileged site. The presence of a blood–brain barrier (BBB) restricts the movement of soluble mediators and leukocytes from the periphery to the

CNS. Despite the presence of this tightly regulated BBB, leukocyte entry into the CNS is an early event in multiple sclerosis (MS). Acute MS lesions, featuring areas of demyelination, axonal loss and immune cell infiltrates, display BBB disruption as evidenced by in vivo gadolinium uptake on magnetic resonance imaging (MRI) and post-mortem evidence of focal micro-vascular leakage. Whether BBB dysfunction precedes immune cell infiltration or is the consequence of perivascular leukocyte accumulation remains to be established. The BCNSB has two further barrier elements: (i) a metabolic barrier that contains a complex array of enzymes (including acetylcholinesterase, alkaline phosphatase, γ-glutamyltranspeptidase, and
Monoamine oxidases) that degrade different chemical compounds thus altering their pharmacological activity and (ii) a transport barrier that contains a variety of efflux transporters, including P-glycoprotein and breast cancer resistance protein [40].

Tight junctions are the critical component of the BCNSB as they control paracellular diffusion and maintain the structural and functional polarity of the specialized endothelial cells of the BBB and BSCB. Thus, the BCNSB contributes to the homeostasis of the parenchyma of the brain and spinal cord and provides protection against many toxic compounds and pathogens[41, 42.]

Indeed, the BCNSB is largely impermeable to compounds that are not lipophilic and have a molecular weight greater than 450 Da. This presents a major

challenge for CNS drug discovery[43, 44]. Early pathologists championed the concept of vascular lesions being an important component of the MS attack. Dawson [45] showed that inflammatory cells clustered around veins in the center of the demyelinated plaque. Putnam and Adler [46] carefully reconstructed serial brain sections from MS patients, and showed that fibrin deposits surrounded the inflamed vessels. Magnetic resonance imaging has confirmed these early pathological studies. Correlation of venous pathology with MS lesions is possible with MR venography, which takes advantage of the difference between oxygenated and deoxygenated blood to visualize venous blood. In most MS lesions, venogram shows a vein in the center of the lesions. This pattern is not specific for MS since a similar pattern with venule involvement occurs with hypoxic ischemic white matter lesions. However, in contrast to MS lesions, ischemic white matter lesions showed no consistent relationship to the shape and location of the veins [46]. A recent study using a 7-T MRI showed that T2*-weighted images can reliably distinguish all patients with clinically definite MS. Small venules in the center of lesions are visualized using T2*-weighted MRI because of the paramagnetic effect of deoxy-hemoglobin. Those patients have >40% of the MS lesions in a perivenous location, while in those without clinical MS, <40% of the lesions appeared perivenous[47.]

2.8 The blood ocular barrier

The blood ocular barrier is composed of the blood-aqueous barrier and the blood-retinal barrier, protecting the eye from entry of toxic substances and maintaining the homeostatic control that underpins the ocular physiology[48, 49].

The blood-aqueous barrier is formed by the non-pigmented epithelium of the ciliary body, the posterior iris epithelium, the endothelium of the iris vessels with tight junctions of the leaky type, and the endothelium of Schlemm's canal.

The blood-retinal barrier consists of the retinal pigment epithelium (outer barrier) and the endothelial membrane of the retinal vessels (inner barrier), both with tight junctions of the non leaky type. The two functional barriers restrict the movement of blood elements to the intraocular chambers [50] and explain why drugs administered orally or intravenously can hardly reach therapeutic levels in intraocular tissues

Endothelial barriers, including the blood-brain barrier (BBB), the blood-retinal barrier (BRB), and the blood-nerve barrier (BNB), shield the nervous system from circulating agents, such as immunoglobulins, that might prove toxic. These barriers also prevent the entry of resting leukocytes from the circulation. Activated T lymphocytes, however, are able to penetrate the barriers through the action of their surface enzymes and adhesion molecules; it is generally

assumed that there are no implications for vascular integrity if there is no antigen recognition in the tissue.

Magnetic resonance imaging of individuals with multiple sclerosis (MS), a relatively common inflammatory demyelinating disease of the CNS, has revealed that breakdown of the BBB is the earliest demonstrable abnormality in the formation of new lesions and in the extension of old lesions. Given that this breakdown of the BBB is thought to play a fundamental role in the pathogenesis of MS, it is important to understand the mechanism by which it occurs. Breakdown of the BBB is always associated with cellular infiltration in individuals with MS.

The retina is an ideal tissue in which to characterize the microvascular and cellular responses of the CNS to an intravascular injection of activated T cells of non-neural specificity, because it is possible to visualize the entire retinal vascular plexus with the normal relations among the glial, vascular, and neuronal elements intact. In particular, with the use of intravascular barrier tracers and cell-specific reagents [51], it is possible to co-localize sites of cellular accumulation with sites of breakdown of the BRB. The retinal whole mount technique has the additional advantage that arteries, capillaries, and venules are readily identified, thereby allowing accurate localization of specific cellular and vascular changes to specific regions of the CNS microvasculature. Taken together, these observations lead us to suggest that microglia are not the cells

that induce the immune response even when the target antigen is encountered; rather, other cells, such as the peri-vascular cells that constitutively express MHC II or circulating monocytes, are responsible for initiation of the immune response.

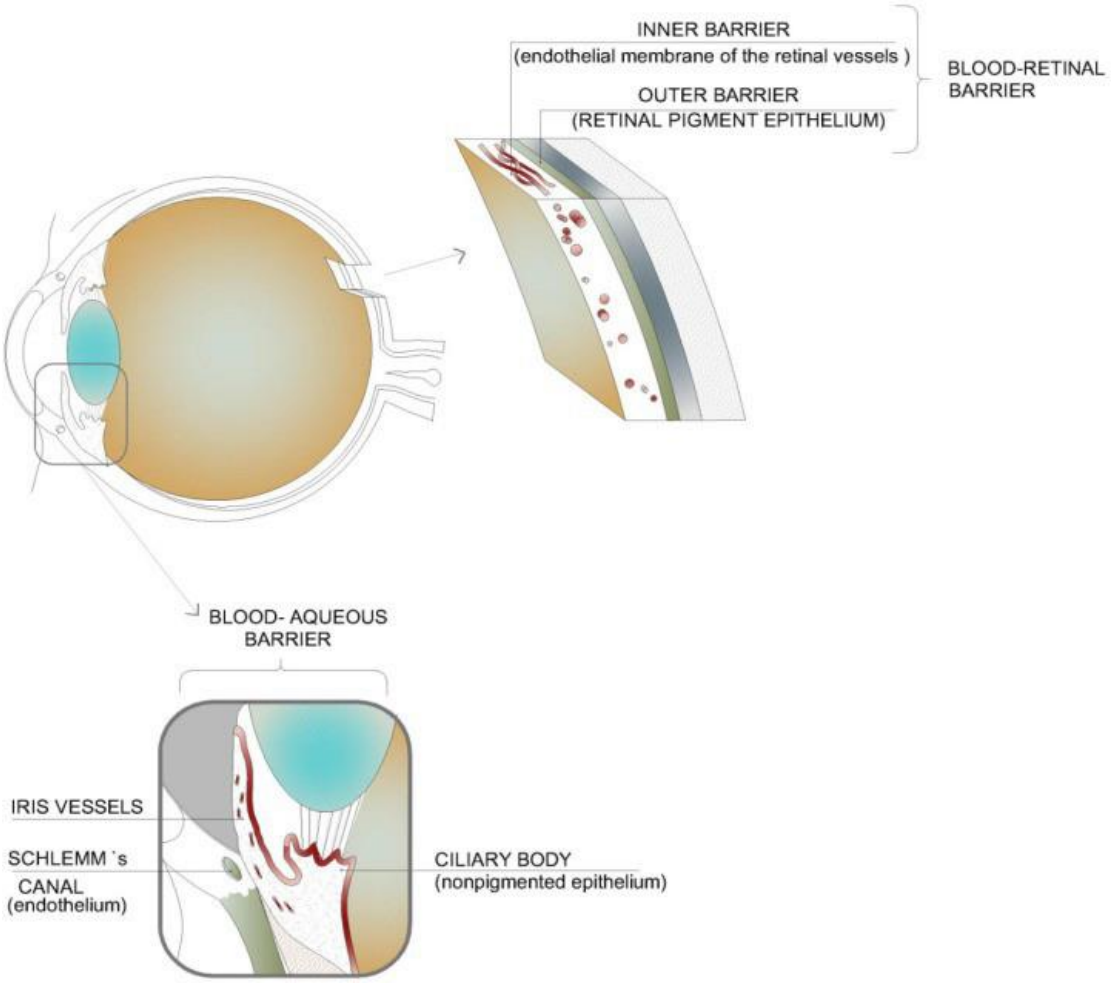


Figure 2.2: Blood ocular barrier (Textbook of Neurology (1997). "The Blood-Brain Barrier in Neuroinflammatory Diseases"**)**

2.9 Recent theory of MS

CCSVI (central venous insufficiency)

In 2009, Italian researcher Paolo Zamboni, first introduced the idea that CCSVI might cause MS [52], or make its symptoms worse. When he used ultrasound to compare the blood vessels of people with and without MS, he found abnormal blood flow in 100% of the people with MS, but in 0% of people without MS. CCSVI stands for chronic cerebrospinal venous insufficiency. It's a narrowing of veins in the neck and chest that carry blood away from the brain and spinal cord. The theory is that when blood flow is slowed, it backs up into the brain and spinal cord and leads to oxygen loss and iron deposits in the brain.

In 2008, two open-minded and forward-thinking MS researchers at the University of Buffalo, Dr Robert Zivadinov and Dr Bianca Weinstock-Guttman, recognized the great potential importance of Dr Zamboni's research and decided to undertake a major study to see if Dr Zamboni's impressive results could be replicated. In 2010 some of the main results of Phase 1 of the Buffalo Study were made public in a press release. These results consist of:

1) 55% of persons with MS in the study had CCSVI

2) 22% of healthy controls had CCSVI

3) 10% of subjects tested (50) were classified as borderline and were included in the non-CCSVI category for the above statistics. When these individuals were excluded from the statistical analysis,

62% of persons with MS had CCSVI and 26% of healthy controls had CCSVI.

4) 38% of those with Clinically Isolated Syndrome (CIS) (start MS in most cases) had CCSVI

5) 80% of those with more advanced MS (EDDS levels not specified) had CCSVI

The new Lancet study [53], including 177 subjects, 79 with multiple sclerosis, 55 siblings, and 43 unrelated controls from three centers in Canada is one of the largest studies of CCSVI and MS. The results of this study are also completely negative – no correlation between CCSVI and MS. They found that catheter venography criteria for chronic cerebrospinal venous insufficiency were positive for one of 65 (2%) people with multiple sclerosis, one of 46 (2%) siblings, and one of 32 (3%) unrelated controls (p=1•0 for all comparisons). Greater than 50% narrowing of any major vein was present in 48 of 65 (74%) people with multiple sclerosis, 31 of 47 (66%) siblings (p=0•41 for comparison with patients with multiple sclerosis), and 26 of 37 (70%) unrelated controls (p=0•82).

It's interesting that about 2/3 of people had greater than 50% narrowing, meaning that such narrowing is a common occurrence and it not necessarily correlated with any disease. This study suggests that Zamboni was simply making an incidental finding of a common benign anatomical variant that is neither pathological nor related to MS.

2.10 Old theories of MS

The cause why the normal appearing areas appear in the brain is unknown. Historically, several theories about how this happens have been presented.

Old blood flow theories

Venous pathology has been associated with MS for more than a century. Pathologist Georg Eduard Rindfleisch noted in 1863 that the inflammation-associated lesions were distributed around veins [54] .Some other authors like Tracy Putnam [55] pointed to venous obstructions.

Some authors like Franz Schelling proposed a mechanical damage procedure based on violent blood reflux [56.] Later the focus moved to softer hemodynamic abnormalities, which were showing precede changes in sub-cortical gray matter [57] and in substantia nigra [58]

Endothelial theory

The various theories such as CSF flow theory, CSF composition, Kir.4 theory and primary neuro-degeneration theories are worth a mention [59]

Iron in MS

MRI and histological studies have shown global alterations in iron levels in the brains of patients with multiple sclerosis (MS), including increases in the iron stored by macrophages and microglia. Excessive free iron can be toxic, and accumulation of iron in MS has generally been thought to be

detrimental. However, iron maintains the integrity of oligodendrocytes and myelin, and facilitates their regeneration following injury. The extracellular matrix, a key regulator of remyelination, might also modulate iron levels. In MS, it is still not clear whether iron deposition is an epiphenomenon or a mediator of disease processes [60].

2.11 Pathology

The pathological hallmark of demyelinating diseases of the central nervous system is characterized by loss of myelin with variable loss of axons [61]. The main demyelinating disease of the CNS is multiple sclerosis (MS) and its variants. Its counterpart in the peripheral nervous system is inflammatory poly-radiculo-neuropathy (Guillain-Barré syndrome-GBS) and its chronic variants. MS and GBS are autoimmune inflammatory diseases. There are also virus-induced demyelinative diseases, such as progressive multifocal leukoencephalopathy (PML). Multifocal lesions, the MS plaques, characterize the pathology. The usual evolution of the MS plaque is as follows: in the acute phase, activated mononuclear cells, including lymphocytes, microglia, and macrophages destroy myelin and, to a variable degree, oligodendrocytes. Myelin debris are picked up by macrophages and degraded. At an early stage, macrophages contain myelin fragments; later, they contain proteins and lipids from chemical degradation of myelin. This evolution takes a few weeks. With time, gliosis develops, and plaques reach a burned-out stage consisting of

demyelinated axons traversing glial scar tissue. Remaining oligodendrocytes attempt to make new myelin. If the inflammatory process is arrested at an early phase, plaques are partially

remyelinated. In more advanced lesions, remyelination is ineffective because gliosis creates a barrier between the myelin producing cells and their axonal targets. The pathological process may be arrested at any time, sometimes after partial demyelination.

The pattern described above is variable. In most cases, the inflammatory reaction subsides only to appear at another location or at another time. Some lesions expand at their periphery while activity in their center dies down. In fulminant MS cases, large lesions with diffuse activity develop and expand inexorably. Although myelin is preferentially affected, axonal loss is significant, and necrosis and cavitation may develop, especially in severe, acute lesions.

Diagnosis of acute MS, especially with stereotactic needle biopsies, may be tricky because cellularity and reactive astrocytes in the lesions may be misinterpreted as a neoplasm. Activity is often confined to the borders of plaques.

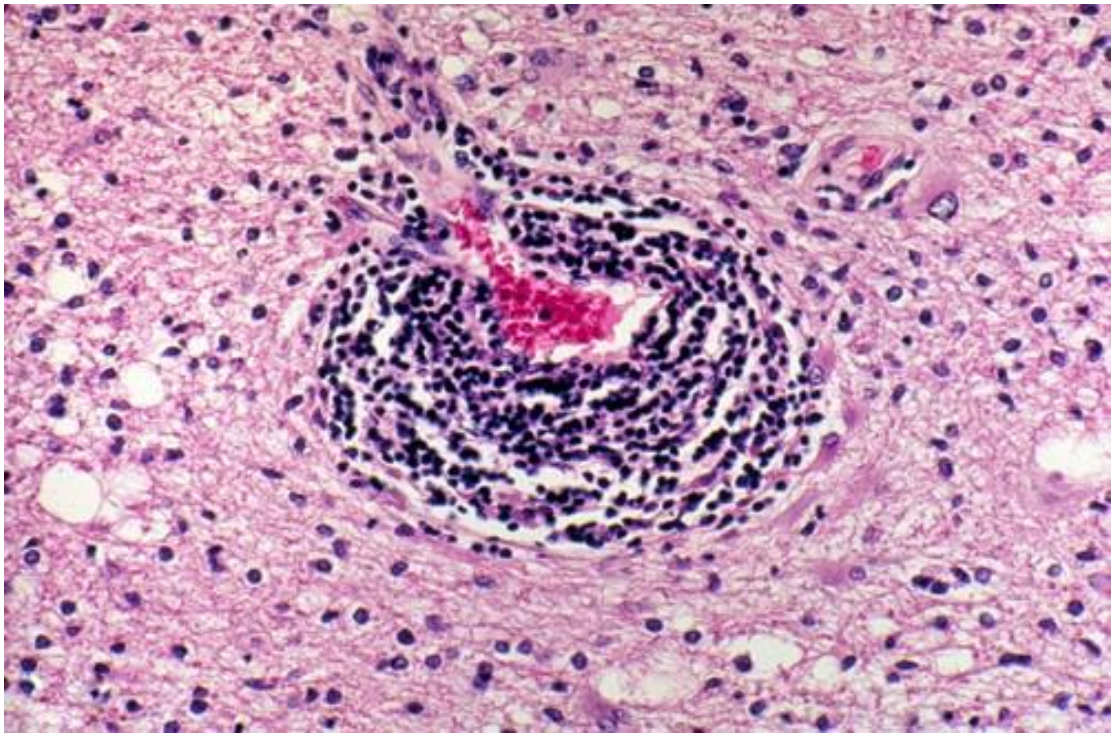


Figure 2.3: Perivascular lymphocytes in an active MS plaque.

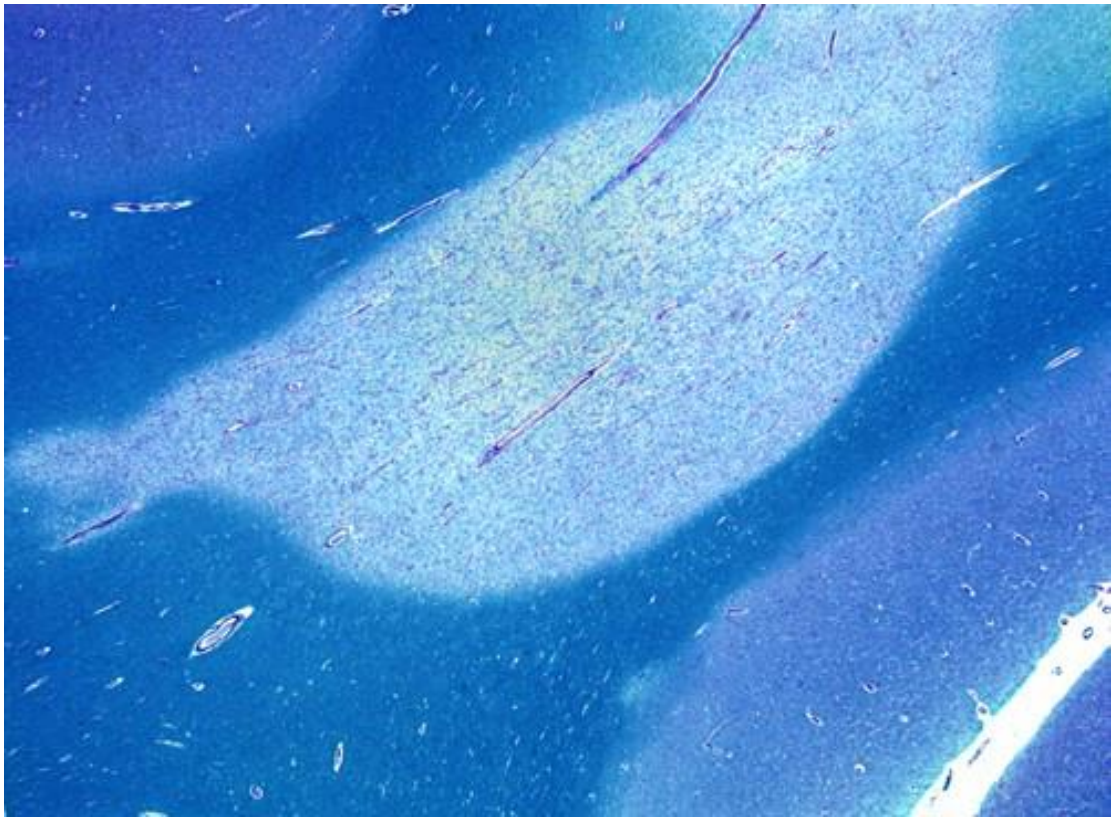


Figure 2.4: Histopathology of MS plaque. Normal myelin stains deep blue.

2.12 Histopathological classification of demyelination

Demyelinating diseases relating to MS in the old system have been classified histologically by pathologist into various groups [62]:

Acute fulminant type (Marburg Type): A very rare form of MS which may be less uncommon than previously thought, first described in 1906 by Otto Marburg. Symptoms may be preceded by fevers, and is typically monophasic. Pathologically shows areas of demyelination with marked macrophage infiltration and extensive axonal damage and necrosis. Extensive confluent areas tumefactive demyelination is seen with mass effect and defined rings and incomplete ring enhancement with rarity of small lesions on brain imaging (MRI). Death from involvement of the brain stem or due to marked mass effect and herniation is common.

Acute disseminated encephalomyelitis (ADEM): An extremely rare progressive demyelinating process that begins in childhood. Symptoms include aphasia, balance instability, dementia, headache, incontinence, personality changes, seizures, tremors, visual disturbances and weakness.

Posner proposed in 1985, all six criteria must be met for diagnosis;

1. One to two large plaques: should be in each brain hemisphere predominantly in centrum semiovale. Lesions are greater than 2 cm in 2/3 dimensions
2. No additional lesions identified

3. No peripheral nervous system abnormality

4. Normal adrenal function

5. Normal serum long chain fatty acids

6. Histological changes consistent with sub-acute/chronic myelino-clastic diffuse sclerosis.

Schilder's type patients have poor prognosis and current recommendations are high dose corticosteroids and supportive care.

Balo concentric sclerosis (BCS) is a rare with presentation closely resembles acute Marburg MS, with rapid progression and sometimes fulminant course. Histopathology reveals concentric layers of alternating demyelination and preserved myelin.

Optico spinal multiple sclerosis (OSMS) is a spectrum of demyelinating disease with similar clinical and imaging features to Neuromyelitis optica (NMO). The identification of anti-aquaporin 4 IgG antibodies in NMO has naturally led to them being sought in patients with OSMS, with varying results, ranging form 35-90%. Whether or not NMO and OSMS are the same disease remains to be fully elucidated.

2.13 Clinical features and differential diagnosis

MS can cause almost any neurological symptom since it can affect any area of the brain, optic nerve, and spinal cord.

Broadly, the initial symptoms include, in decreasing order of frequency:

• Weakness in one of more limbs

• Blurred vision relating Optic/ retrobulbar neuritis

• Tingling and numbness (paraesthesia)

• Double vision (diplopia), nystagmus

• Vertigo

• Disturbance of micturition

• Spasticity,

• Fatigue

• Pain

• Uhthoff sign: transient impairment of any function due to increase body temperature due to exercise, hot bath, hot weather.

• Lhermitte's sign

• Mental changes including depression, dementia, and memory loss

With the diversity in symptoms, signs and clinical course MS provides a source of misdiagnosis for even the experienced clinicians.

2.14 Clinical classification of MS

They are important not only for prognosis but also for treatment decisions. In 1996, the United States National Multiple Sclerosis Society described four clinical courses [63.] An international panel later reviewed this set in 2013, adding clinically isolate syndrome (CIS) and radiologically isolate syndrome (RIS) as phenotypes. Patients usually experience a first neurologic event suggestive of MS known as Clinically Isolated Syndrome (CIS). It lasts for at least 24 hours, with symptoms and signs indicating either a single lesion (monofocal) or more than one lesion (multifocal) within the central nervous system [64].

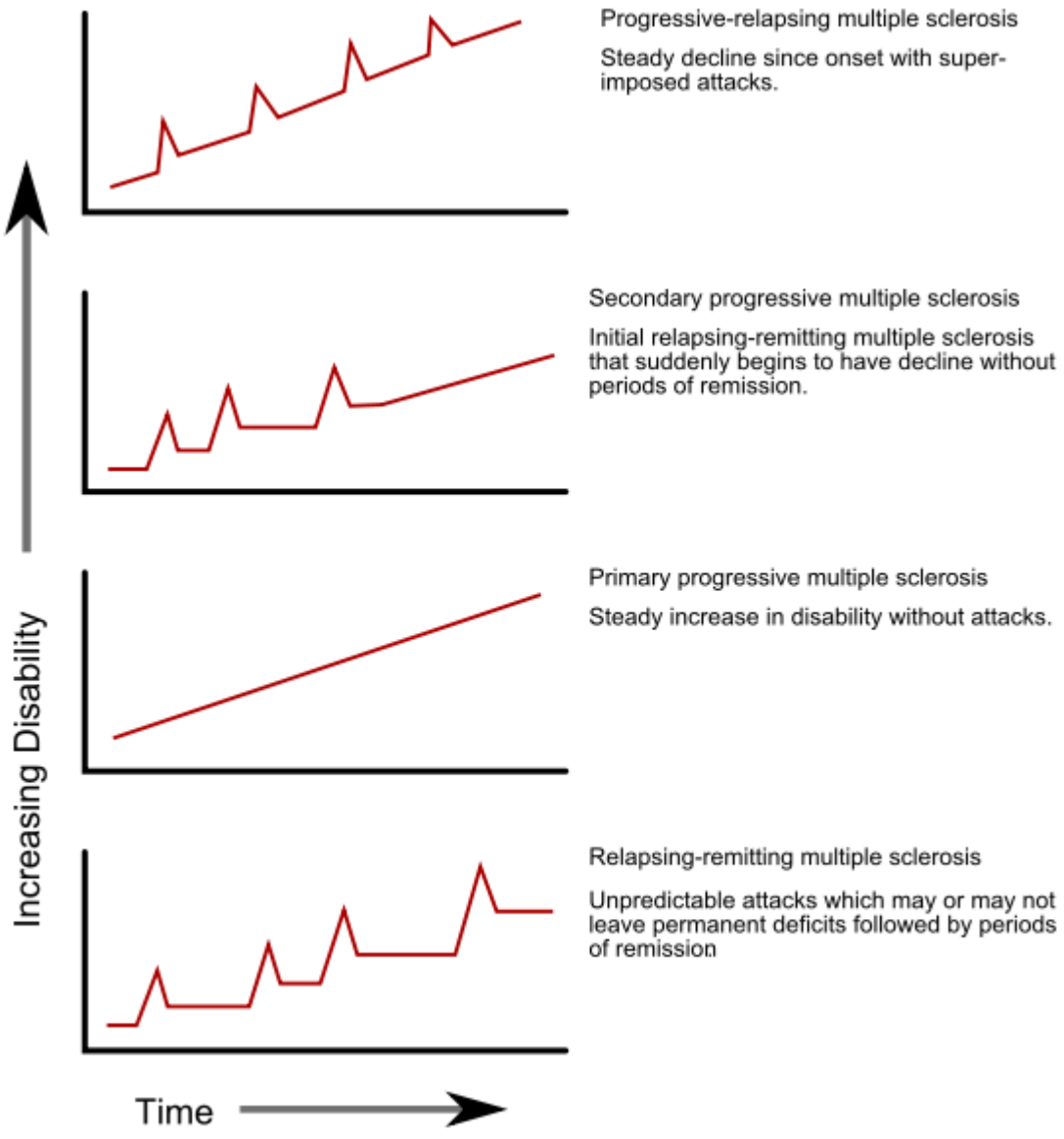
The four clinical courses are

- Relapsing-remitting (RRMS)
- Secondary progressive (SPMS)
- Primary progressive (PPMS)
- Progressive relapsing.

The relapsing-remitting course is the most common and carries the best prognosis. However, although symptoms remit, there is usually a mild residual deficit. Over time, these mild deficits produce disability. After about 6 to 10 years, approximately 30% to 40%of cases of relapsing-remitting MS evolve into a progressive form (secondary progressive).

In the progressive form, symptoms worsen insidiously without a clear period of remission. Relapses may be superimposed on a background of functional decline. In the primary progressive form, there are no periods of remission. Instead, steady progression of symptoms leads to disability from the start.

Table 2.6: Illustration of different clinical courses of multiple sclerosis. Adapted from Lublin FD, et al.



2.15 Diagnosis of MS

There is no pathognomonic clinical, laboratory, or imaging finding in MS. The diagnosis ultimately is a clinical decision based upon weighing[65] the factors that support the diagnosis against those that fail to support it or point to the possibility of an alternative diagnosis [66](NMO and OSMS).

The Schumacher criteria/ Poser from 1965 capture the essence of the diagnosis of MS: CNS lesions disseminated in space and time, and the elimination of alternative diagnosis. The Schumacher criteria required an age range between 10 and 50 years and objective abnormalities on examination, which are now outdated. However, the main concepts

captured by these criteria remain relevant today.

The criteria from the International Panel on MS Diagnosis, also called the McDonald Criteria, are a set of diagnostic criteria for MS that incorporate the clinical characteristics alone or in combination with MRI features.

10 Revisions were made in 2005 and again in 2010 as a reflection of an increased understanding of the natural history of MS revealed by the MRI and clinical progression, and improved MRI techniques. The latest version of the McDonald Criteria (2010) has simplified the diagnostic process, allowing MS to be diagnosed earlier (Table 2.7)

Table 2.7: 2010 Revised McDonald Criteria for the Diagnosis of Multiple Sclerosis

Clinical Presentation	Additional Data Needed for MS Diagnosis
• Two or more attacks	None; clinical evidence will suffice. Additional evidence (e.g., brain MRI) desirable, but must be consistent with MS
• Objective clinical evidence of 2 or more lesions with reasonable historical evidence of a prior attack	
• Two or more attacks	Dissemination in space demonstrated by MRI or Await further clinical attack implicating a different site
• Objective clinical evidence of 1 lesion	
• One attack	Dissemination in time demonstrated by MRI or second clinical attack
• Objective clinical evidence of 2 or more lesions	

• One attack • Objective clinical evidence of 1 lesion (clinically isolated syndrome) · Insidious neurologic progression suggestive of MS	Dissemination in space demonstrated by MRI or await a second clinical attack implicating a different CNS site and Dissemination in time, demonstrated by MRI or second clinical attack One year of disease progression and dissemination in space, demonstrated by 2 of the following: • One or more T2 lesions in brain, in regions characteristic of MS • Two or more T2 focal lesions in spinal cord • Positive CSF	

Notes: An attack is defined as a neurological disturbance of the kind seen in MS. It can be documented by subjective report or by objective observation, but it must last for at least 24 hours. Pseudo attacks and single paroxysmal episodes must be excluded. To be considered separate attacks, at least 30 days must elapse between onset of one event and onset of another event.

After a single episode of demyelination, certain findings on one MRI can help fulfill the diagnostic criteria for MS, even before a second clinical episode or new MRI lesion. The revisions also preserve diagnostic sensitivity and specificity[67] and address their applicability across different populations, allowing for more uniform and widespread use across groups.

2.16 Conditions that mimic MS and may be considered in the differentials[68]

Diseases capable of causing multiple lesions of CNS often causing relapsing and remitting course

- Systemic Lupus erythematosus
- Primary Sjogren’s Syndrome
- Polyarteritisnodosa
- Behcets disease
- AIDS
- Sarcoidosis
- Devic's disease (NMO)
- Lyme's disease
- Sub-acute myeloencephalitis
- Cerebrovascular disease
- Meningovascular syphilis
- Remote effects of carcinoma

2 Systematised diseases

- Hereditary spino-cerebellar ataxia

- Subacute combined degeneration of Spinal cord

- Leukodystrophy

3 **Single lesion of central nervous system with a relapsing and remitting course**

- Tumours

- Arterio-venous malformations

4 **Single lesion of central nervous system with a progressive course**

- Tumours

- Cervical spondylotic myelopathy

- Chiari Malformations

5 **Mono-symptomatic presentations**

- Visual failure

- Diplopia

- Sensory symptoms

6 **Non organic symptoms**

2.17 Management of MS

Multiple sclerosis is a complex disease and management is mainly led by a multidisciplinary team of consultant neurologists, MS nurses, physiotherapists and occupational therapists, speech and language therapists, psychologists, dietitians, social care and continence specialists and General practitioners. Management of MS requires a constant counselling and update to the affected and their family members in addition to the medical management of the condition.

Medical management can be divided into 2 categories:

a) Treat acute symptoms with steroids

NICE recommends use of intravenous and oral steroids for acute relapses. 1gram IV methyl prednisolone is given for 3 days for confirmed relapses. Use of steroids is controversial as recent research evidence suggests a delay in re-myelination process and a long term build-up of disability. On the contrary, it is not clear if untreated, the patients would still accumulate disability. Other treatment for acute relapses includes immunoglobulins, plasma exchange and ACTH (Adrenocortico trophic hormones).

b) Long term medical management using Disease modifying therapy (DMT) along with symptom management and rehabilitation.

Long-term treatment with disease modifying agent is made after careful consideration of disease stage, tolerance, anticipated adverse effects and life

style. Disease-modifying agents aim to slow the progression of the disease, reduce accumulation of lesions in the brain and spinal cord as measured by MRI and also reduce acute attacks.

The DMT's currently in use are:

a) Interferons: Interferon beta-1b (beta interferon)[67] and Interferon beta-1a (Avonex, Rebif)

Mechanism of action: The exact mechanism of action is unknown, but they inhibit the pro-inflammatory cascade including gamma interferon .

Caution**:** Patients with uncontrolled depression.

Adverse effect beta interferons: Include flu-like symptoms, elevated liver enzymes, and injection site reactions.

Adverse effects Rebif: Fewer flu-like symptoms. Other adverse effects include hepatic and hematologic complications, which are usually asymptomatic.

b) Fingolimod (Gilenya):

Mechanism of action: First oral treatment which modulates a subset of G-protein receptors leading to B lymphocyte sequestration in the lymph nodes and reduces their migration in the nervous system.

Adverse reaction: Fingolimod is associated with bradycardia and prolonged QT syndrome.

Caution: Absolute lymphocyte count needs to be monitored which can be a major drawback

c) Natalizumab (Tysabri) [68]:

This monoclonal antibody binds to α-4 integrin and inhibits binding to its receptors. This monotherapy delays physical disability and decreases frequency of acute attacks. Natalizumab is associated with progressive multifocal leukoencephalopathy and is only available via a restricted prescribing program called TOUCH. Standard dose for the IV infusion is 300mg over one hour every four weeks.

d) Glatiramer acetate (Copaxone) [18]:

This is a polypeptide involved in immune process modification in the pathogenesis of MS. It has been shown to decrease the acute exacerbation rate by 29% in patients with RRMS and is shown to reduce their frequency. However, it is used as an FDA approved second line of treatment in RRMS in patients showing convincing changes on the MRI .

e) Daclizumab and Ocrelizumab: These are monoclonal antibodies currently being studied that also have shown promise for patients with MS.

f) Cladribine, Laquinimod [69]: These are the few new agents under investigation.

Chapter 3: The visual system in Multiple Sclerosis

3.1. Introduction

The human visual system relies on a constant and rapid flow of information along the visual pathway. The complex visual system can be delineated into 2 major pathways, based on the presence and absence of myelin sheath.

a) The pre -lamina cribrosa pathway is composed of un-myelinated nerve fiber, extending from the retinal ganglion cells (RGC), both peripheral retina and central macular retina, to its entry into the optic nerve head (ONH) until it reaches the lamina cribrosa (LC).

It is rich in neurons and axons of the ganglionic cells (90%) along with supporting Muller and glial cells.

b) The post lamina cribrosa pathway is composed of myelinated nerve fiber, extending beyond the lamina cribrosa and includes the optic nerve (ON), optic chiasm (OCH), optic tract (OT) and visual cortex (VC).

It is a mixture of myelin and axons as it is with the rest of the central nervous system

3.2. Eye "the window to the brain" : Philosophers defined the eye as a "window to the soul" long before scientists addressed this cliché to determine its scientific basis and clinical relevance. Anatomically and developmentally, the eye is an extension from the brain. The retina axons are in effect the CNS axons. The eye is the only part of the brain that can be seen directly via the optic nerve and the retina, which makes the eye unique [70]

3.3. Eye "a model of neuro-degeneration in MS": The anterior visual system is a frequent target of the MS disease process that almost all patients with MS[71] are found to have characteristic MS changes in the retina and optic nerve, regardless of whether they have previously experienced acute optic neuritis (AON) [72, 73]—although as many as 30–70% of patients with MS will, in fact, have inflammatory optic neuritis during the course of their disease [74]. The pathophysiology of multiple sclerosis (MS) is characterized by demyelination, which culminates in a reduction in axonal transmission. Axonal and neuronal degeneration seem to be concomitant features of MS and are probably the pathological processes responsible for permanent disability in this disease. The retina is unique within the CNS in that it contains axons and glia but no myelin, and it is, therefore, an ideal structure to visualize the processes of neurodegeneration, neuroprotection, and potentially even neurorestoration.

In particular, the retina enables us to investigate a specific compartment of the CNS that is targeted by the disease process [72]

3.4. Visual Symptoms in MS

MS affects the visual system in a large proportion of patients to an extent that visual symptom is the first presenting feature in 15-20% of the patient and 50% of the patients have visual problems during the course of the disease. With visual system gaining importance in diagnosis and management of MS, it has become evident that asymptomatic/ sub-clinical visual loss is a classical feature in MS. This in-turn affects quality of vision and life in MS.

The Ophthalmic manifestations of multiple sclerosis can be divided into two main categories: those that affect the visual sensory system and those that affect the ocular motor system.

Visual sensory system involvement in the order of occurrence:

1) Optic neuritis

Demyelinating optic neuritis can be considered in three categories:

a) Acute

b) Chronic

c) Sub-clinical (asymptomatic)

2) Choroidal and retinal lesions

3) Posterior uveitis that includes branch retinal occlusion, neovascularization, retinal venous sheathing

4) Intermediate Uveitis (Pars Planitis)

Pars planitis is a condition characterized by intraocular inflammation consisting of cells and debris in the vitreous, condensation of the vitreous along the pars plana, and varying degrees of periphlebitis. There is usually little or no inflammation in the anterior chamber. The condition can have numerous sequele that may threaten vision, including cataract formation, development of epiretinal membrane in the macula, and cystoid macular edema. Malinowski et al [75] found that 6.2% of patients with pars planitis were at risk of developing MS. Malinowski also found a strong association of pars planitis with HLA--DR2 and the temporal development of MS . Genetic predisposition in MS has always been a critical concern in aetiology and progress of the disease. Kheradvar et al [76] presented relations between human leukocyte antigen (HLA), optic neuritis (ON) and MS in the Iranian population. These studies strongly suggests the association among DR2, A23 and B21 allele and the evolution of ON to MS. High prevalence of A23 and DR2 alleles in clinically definite MS patients compared with the normal population may suggest an important role for these alleles in the development of MS. The study suggests B51 as a protective factor against development of ON in the normal population

3.5 Oculomotor system involvement:

Patients with multiple sclerosis may develop disorders of fixation, ocular motility and ocular alignment. Nystagmus is the most common disorder of fixation associated with MS, including upbeat nystagmus, downbeat nystagmus, see-saw nystagmus, periodic alternating nystagmus and convergence-induced pendular nystagmus[77]

Various saccadic intrusions – inappropriate saccades hat interfere with fixation – may occur in patients with multiple sclerosis, including ocular flutter, opsoclonus,and square-wave jerks. Clinical examination of eye movements, with attention to dynamic properties of saccades and the vestibulo-ocular reflex, takes only a few minutes to perform, but may provide better information concerning the presence of brainstem and cerebellar involvement[78,79]. Disturbances of ocular motility or alignment may develop during the course of MS, usually result from demyelinating lesions in the brainstem that affect supranuclear, internuclear, nuclear, or fascicular pathways[80].

Frohman etal[81] compared the accuracy of clinical detection of internuclearophthalmoplegia (INO) with that of quantitative infrared oculograpy and found good detection rates with clinical examination.

3.6. Optic neuritis:

Introduction

Optic neuritis refers to the inflammation of the optic nerves. There are many different causes though it is commonly associated with multiple sclerosis. Other causes include vascular, metabolic, hereditary, systemic disease, focal compressive lesions and toxins.

In the absence of prior or concomitant neurological symptoms, then the ON may be referred to as idiopathic, mono-symptomatic or isolated. ON usually determines a moderate to severe visual impairment, followed by a complete or near complete clinical recovery within a few weeks.

The process of recovery of vision has been based on two hypotheses, which do not exclude each other. The first suggests that axons of the optic nerve can maintain a normal clinical function up to a critical threshold of nerve fibers loss, as a result of the intrinsic structural reserve (also known as neuroaxonal redundancy) [82]. The second supports the role of adaptive functional changes taking place at the level of striate and extra-striate visual cortical areas. The repair and remyelination process has been related to sodium influx across the cconducting axon in recent studies by Franklin and his group from Cambridge University.

3.7. Anatomical Classification[83] of Optic neuritis:

Papillitis

It is the inflammation of optic nerve head with evident disc swelling is the usual presentation. It affects the paediatric age group and is mostly bilateral.

Retrobulbar optic neuritis

It is the inflammation of the optic nerve beyond the lamina cribrosa. Blurred vision, loss of color vision and pain on eye movement is the classic triad. Pain is mainly attributed to the stretching of the optic nerve sheath during eye movements.

3.8. Clinical classification:

A condition causing a relatively rapid onset of visual loss with spontaneous recovery within 3 weeks to 3months is termed acute and those that are symptomatic beyond 3 months are termed chronic optic neuritis.

Inflammation of the single nerve is termed acute unilateral ON (AON) while inflammation of both optic nerves is termed bilateral ON (BON). There are 2 forms of BON depending on the time course over which the two eyes get affected. If the second eye develops ON within a three week to a three month period then the disease may be considered simultaneous (BSimON). This is much rarer than bilateral sequential disease (BSeqON) where the fellow eye is

affected at-least 3 months after the first and often after a far greater time than this. Sequential disease is not uncommon in MS when etiologies other than demyelination are frequently responsible.

Typical acute optic neuritis (AON**)**

- Young adult patient <50 years old
- Acute or sub-acute visual loss
- Progressive over a few days up to 2 weeks
- Unilateral visual loss with reduced colour and contrast vision
- Peri-ocular pain and painful eye movement
- Normal or swollen optic disc
- Normal macula and peripheral retina

Features considered atypical for demyelinating optic neuritis

- Aged <15 years or >50 years
- No relative afferent pupillary defect
- Aquaporin-4 (neuromyelitisoptica) antibody positivity
- Immediate and dramatic response to steroids
- Bilateral or chiasm involvement
- Severity – no light perception or hand motion vision
- Steroid dependence (worsening of vision with steroid tapering)

3.9. Phenotypic classification:

SION- single isolated optic neuritis

RION - relapsing isolated optic neuritis

CRION- chronic relapsing inflammatory optic neuropathy (CRION),

NMO and OSMS spectrum disorder

MSON -multiple sclerosis associated optic neuritis

UCON- unclassified optic neuritis (UCON) forms

Other causes of AUON and BON

- Unknown
- Ischaemic
- Compressive
- Infiltrative
- Traumatic
- Mitochondrial
- Hereditary
- Toxic
- Nutritional

3.10. Diagnostic Criteria of Optic neuritis (ICD-10)

1. Visual impairment due to a central or paracentral scotoma
2. Onset of pain and onset of visual impairment separated by <4 weeks
3. Impaired colour vision
4. Relative afferent pupillary defect
5. Optic disc swelling
6. Pain resolves within 4 weeks
7. A compressive lesion has been ruled out.

Chapter 4

The visual pathway

4.1. Structure of the retina and optic nerve head

Introduction:

Multiple sclerosis and its pathological process became more established from 2006 onwards. In this process of demyelination, the evident axonal damage and neuronal loss was probably contributing to accumulation
of permanent disability affecting patients and their quality of life. The concept of "eye being a window to the brain" became more acceptable as retina was considered unique. Unique, because the retinal ganglion and axons were not only devoid of the myelin sheath, but also was easily visualised for assessment of changes.

The unmyelinated nerve fiber seemed a perfect pitch to study the process of neurodegeneration, neuroprotection, and potentially even neuro- restoration[84].

4.2. Structure of retina: The anatomical pitch

The retina is a multi-layered sensory tissue[85] that lines the back of the eye, in front of the choroid and sclera and form the starting point for receiving and processing the information. It contains millions of photoreceptors that capture light rays and convert them into electrical impulses. These impulses travel along the optic nerve to the brain where they are turned into images.

There are two types of photoreceptors in the retina: rods and cones.

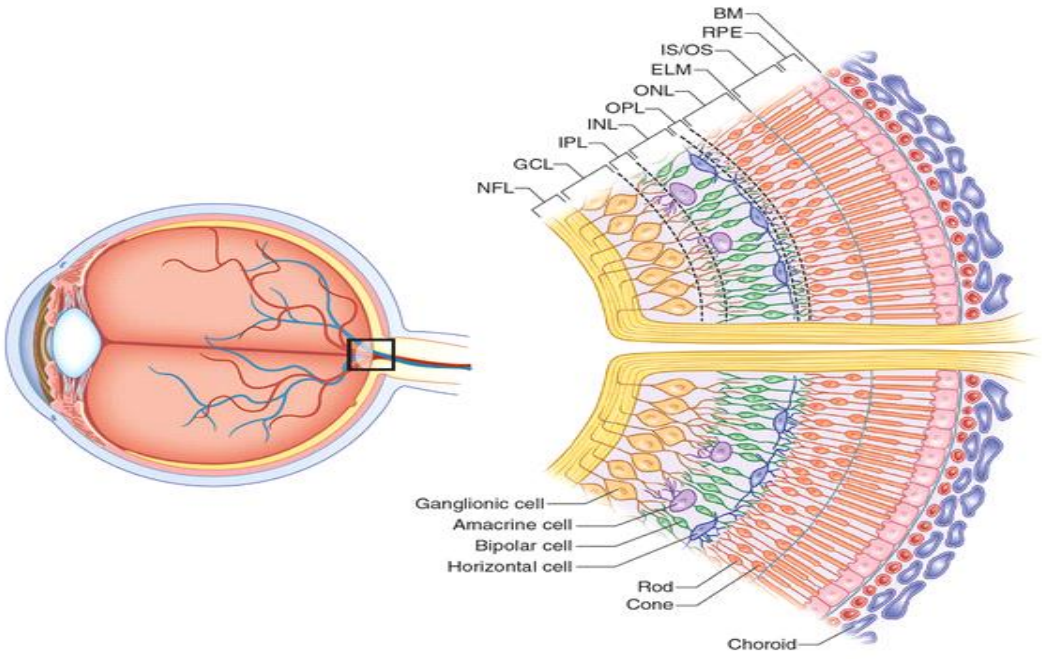
Cones: The retina contains approximately 6 million cones. The cones are contained in the macula, the portion of the retina responsible for central vision. They are most densely packed within the fovea, the very centre portion of the macula. Cones function best in bright light and allow us to appreciate colour.

Rods: There are approximately 125 million rods. They are spread throughout the peripheral retina and function best in dim lighting. The rods are responsible for peripheral and night vision.

Retina:

The retina[86] is a complex multi-layer functional unit. **See Fig** 4.1

Figure 4.1: Structure of the Retina



Embryologically the retina develops from the optic vesicle, which is invaginated by the ectodermal placode. This invagination gives rise to the multi layered inner neuro sensory retina and a monolayer of outer retinal pigment epithelium. The hyaloid circulation supplies the embryonic retina, which is then taken over by the retinal artery and vein in the 5^{th} month of pregnancy. The final adult retina pattern is achieved at birth. The optic nerve becomes progressively myelinated from the proximal to the distal direction, in most cases stopping at the lamina cribrosa. This contributes to a normal transparent retina.

Retinal layers (outer to innermost) are:

1. Pigment epithelium (RPE)
2. Photoreceptor with its Outer and Inner Segment Layers (OS & IS)
3. External Limiting Membrane (ELM)
4. Outer Nuclear Layer (ONL)
5. Outer Plexiform Layer (EPL)
6. Inner Nuclear Layer (INL)
7. Inner Plexiform Layer (IPL)
8. Ganglion Cell Layer (GCL)
9. Nerve Fibre Layer (NFL)
10. Internal Limiting Membrane (ILM)

1. The Retinal Pigment epithelium (RPE)

The RPE is made up of cuboidal cells, which contain the two pigments, melanin and lipofuscin. This layer is firmly attached to the choroid through the Bruch's membrane.

The functions of RPE are:

– Remove water and maintain ionic microenvironment

– Nourish the outer parts of the retina

– Phagocytose detached discs from the rod and cone outer segments. Shed discs from the outer rod segment are enclosed by delicate cytoplasmic extensions of the pigment epithelium (PE) and later broken down.

2. Photoreceptor outer and inner segment layers (OS & IS)

Cones and rods form the vital layer, cones functioning during the day, assisting colour vision and rods assisting in dark adaptation and night vision. Cones are highly concentrated in the fovea of the macular area and aid central vision. Rods density is highest approximately 20degrees outside the fovea and serve high acuity for peripheral vision.

Both the outer segment and inner segment of the rods and the cones are connected through the cilium and forms a transmission channel. The outer segments are composed of approximately 2000 discs stacked upon each

other. They contain visual pigment proteins, Rods containing Rhodopsin and cones containing Opsin tuned to long, medium and short wavelengths. It must be remembered that there is an age related receptor loss in the retina in normal individuals and this loss is accelerated in pathological conditions.

3. External Limiting Membrane

This is not a true membrane but is made up of an array of desmosomal contacts between Muller fibres and receptor cells, producing a pattern of netting. The Muller cells have their nucleus in the inner nuclear layer. They are large complex neuroglial cells stretching from ILM to just beyond ELM. Muller cells plays importance in breakdown of glutamate, providing an environment of homeostasis of the ionic micro-environment and neuronal survival.

4. Outer nuclear layer (ONL)

The ONL is mainly composed of the cell bodies of the rods and the cones with their nuclei and cytoplasm .The Rod nuclei are round/oval while the Cone nuclei are oval.

5. Outer plexiform layer (OPL)

OPL is made of axons of the rods and cones. They contact processes from the bipolar and horizontal cells that are also found in this layer. The synaptic

endings of the rods and cones are known
as spherules and pedicles respectively. There are two main types of cone to bipolar cell contact, Synaptic Ribbon Synapses and Basal (or Flat) junctions' contacts. Horizontal cells have much wider spread of processes and contact more photoreceptors than bipolar cells and mediate lateral interactions. Bipolar cells convey visual signals vertically from outer to inner plexiform layer.

6. Inner Nuclear Layer (INL)

• Horizontal cells, Muller cells, Bipolar cells, interplexiform cells
and amacrine cells all have their nuclei in this layer. This layer is thickest in the fovea, thinning towards the ora serrata.

7. Inner Plexiform Layer (IPL)

The IPL is thicker than outer plexiform layer (18-36μ) as at this layer
the second and third order neurons of the retina meet. In this layer,
bipolar, amacrine, inter plexiform and ganglion cells form synapses. Bipolar cells release neurotransmitter, glutamate. They synapse
with amacrine cell processes and ganglion cell dendrites. Amacrine cells inter-relate adjacent areas of retina that conduction can be both directions suggesting that a local feedback system exists.

8. Ganglion cell layer (GCL)

This layer is 1-2 cells thick (10-20 μ) except at macular region where it thickens to 80 μ or more

There are 3 major types of ganglion cell:

a) Midget – Narrow dendrite tree -70% of all ganglion cells in central retina

b) Parasol – Vary in size and shape – more common in peripheral retina and contribute to 10% of all ganglion cells

C) Small bi-stratified – receive input from blue cone and bipolar cells – about 10%

9. Retinal Nerve Fibre Layer (RNFL)

This layer is 20-30μ at the optic disc and consists of the axons of ganglion cells, which radiate towards the optic nerve head in characteristic fashion. At this point they will form the optic nerve and become myelinated at lamina cribosa.

10. Inner Limiting Membrane (ILM)

The ILM is 1-2microns thick and very different from External Limiting Membrane. It is absent at the optic disc

4.3. Retinal Modifications

FOVEA

The main mentionable modification of the retina[86] is the very centre of the retina called fovea.

At the fovea, the layers of the retina are excavated. The foveola is the most central part of the fovea and is completely rod and blue cone free. Approximately 10,000 are found at the foveola. The foveal pit acts as a mirror and one can see the reflex from it when using an ophthalmoscope. Neither the choriocapillaries nor the RPE are modified at the fovea. The cones at the foveola are much longer and thinner than elsewhere. The smaller diameter of foveal cones allows more of them per unit area give better resolution. A yellow pigment, Xanthophyll acts as a yellow filter absorbing blue light and making this area blue blind

Optic nerve head (ONH)

The optic nerve head is the beginning of the optic nerve and forms a primary portal of collecting visual information from the retina to the brain. The nerve also provides an entry and exit point into the eye for the central retinal artery and central retinal vein that provides nourishment for the superficial layers of the retina.

The ONH is composed of the axons of ganglion cells in the retina. Approximately 1.2 million axons converge to form the optic nerve head that

exits the eye through a sieve-like structure in the sclera called the lamina cribrosa. There are no photoreceptors at the optic nerve head, and so it projects as a physiological blind spot. Ordinarily, we do not notice this blind patch in our vision owing to it overlapping with an area of normal sight in the fellow eye, and the tendency of our visual system to fill in gaps, rather than perceive them as black patches. Within the retina the axons pass naked along its surface, whereas after exiting the eye they become enveloped in myelin sheaths so as to promote the propagation of electrical signals. If the axons were myelinated within the retina they would appear white and prevent light from passing to the underlying photoreceptors. Viewed en-face the optic nerve head is seen as a circular disc with a small depression in the centre referred to as the optic cup, to contrast it with the surrounding neural tissue that is referred to as the neuroretinal rim. The cup-to-disc ratio is important clinically because its increase in the cup disc ratio indicates death of ganglion cells

4.4. Beneath the retina:

Beneath the retina, resting on the retinal pigment epithelium is the choroid. Choroid is a highly vascular layer and forms the middle coat (tunic) of the eye. It extends from the margins of the optic nerve to the pars plana, where it continues anteriorly, becoming the ciliary body.

Histologically, the structure of the choroid is generally divided into four layers (classified in order of furthest away from the retina to closest):

- Haller's layer - outermost layer, consisting of larger diameter blood vessels
- Sattler's layer - layer of medium diameter blood vessels
- Choriocapillaries - layer of capillaries, and
- Bruch's membrane - innermost layer of the choroid. This forms the Bruch’s RPE complex that is the outer retinal barrier and plays a major role in autoimmune diseases.

Interestingly choroid is embryologically similar to the choroid plexus and thus study of choroid, is an area of interest in this research project.

Chapter 5

Structural assessment of visual system

5.1. Structural assessment: "The more than meets the Eye"

Multiple sclerosis is a progressive disease with accumulation of disability over a period of time. There is a process of demyelination compounded by axonal degeneration leading to irreversible damage.

Clinical assessment and clues from MRI have been used until date to study the rate of progression. MRI is helpful to determine activity and lesion load. On the contrary there is an element of clinical–radiological disassociation that means that a patient may be asymptomatic, even with multiple lesions in the brain and vice versa.

The only part of the brain that can be visualised is the retina and optic nerve, thus, making eye "a window to the brain". With advent of OCT, the visualised part of the brain can be quantified. This in-turn helps to determine rate of progression, response to disease modifying treatment and overall generate a model of neurodegeneration in multiple sclerosis.

Above all, the OCT is a non-invasive investigative technique and takes only a 1-2 minute to perform the scan. The principle of coherence interferometry means that there is no radiation to the patient.

5.2. Optical Coherence Tomography (OCT)

"A window into the mechanisms of multiple sclerosis"

In 1851, Von Helmholtz[87] introduced the use of hand –held ophthalmoscope to visualise the retina and optic nerve head. In 1974, Frisén and Hoyt [88]reported the thinning of retinal nerve fiber using the red free filter in the Ophthalmoscope .In the recent years quantitative ophthalmoscopes that can be used efficiently and objectively to measure changes in structural architecture within the retina has gained importance.

Quantitative ophthalmoscopes in current use are

- Optical Coherence Tomography (OCT)
- Heidelberg Retinal Tomography (HRT)
- Scanning Laser Polarimetry (SLP) with variable corneal compensation (GDx -VCC)

5.3. The advent of OCT:

OCT was first reported by Huang-et-al. In 1991[89]

In-vivo retinal imaging was first demonstrated in 1993[90, 91]

Early studies in 1995 provided the first demonstration of OCT imaging of the normal retina[92.]

Spectral domain and high speed and high resolution OCT was reported in 2002 and 2003[93, 94, 95]

OCT became commercially available in 2006.

5.4. Principle of OCT:

The principle of OCT is white light or low coherence interferometer. The optical set up typically consists of an interferometer (Fig5.1, typically Michelson type). Light is split into and recombined from reference and sample arm, respectively. Assembling a collection of neighboring A-scans produces a cross-sectional image.

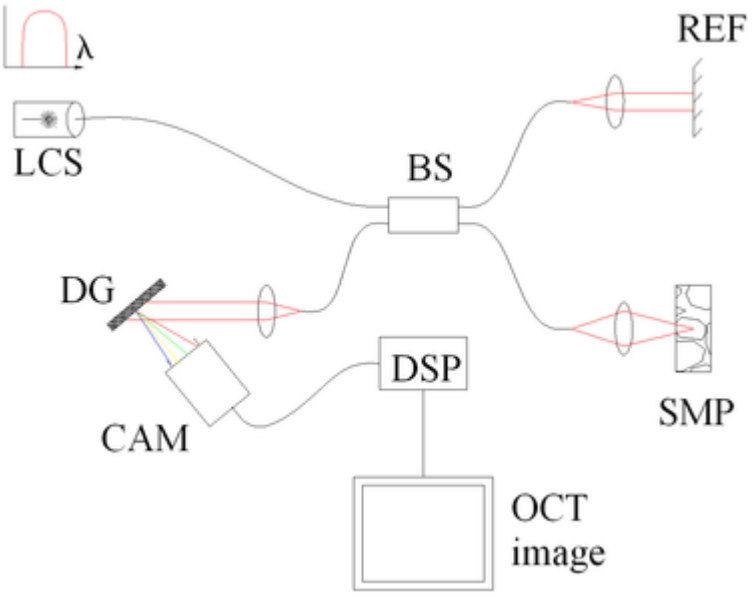


Figure 5.1: Principle of Spectral domain OCT. Components include: low coherence source (LCS), beamsplitter (BS), reference mirror (REF), sample (SMP), diffraction grating (DG) and full-field detector (CAM) acting as a spectrometer, and digital signal processing (DSP)

5.5. Spectral domain OCT (Spectralis): SD-OCT extracts spectral information by distributing different optical frequencies onto a detector stripe through a dispersive element. Thereby the information of the full depth scan can be acquired within a single exposure. The volumetric image is then constructed from a collection of B-scans. There are three major types of volumetric images used in OCT imaging:

- Rectangular, or raster volume scan: A series of parallel B-scans
- Radial volume scan: A series of B-scans at regular angular intervals
- Annular volume scan: A series of B-scans forming concentric rings

Figure 5.2, Figure 5.3 and Figure 5.4 shows the histological retina to correlate with findings on Ocular Coherence Tomography.

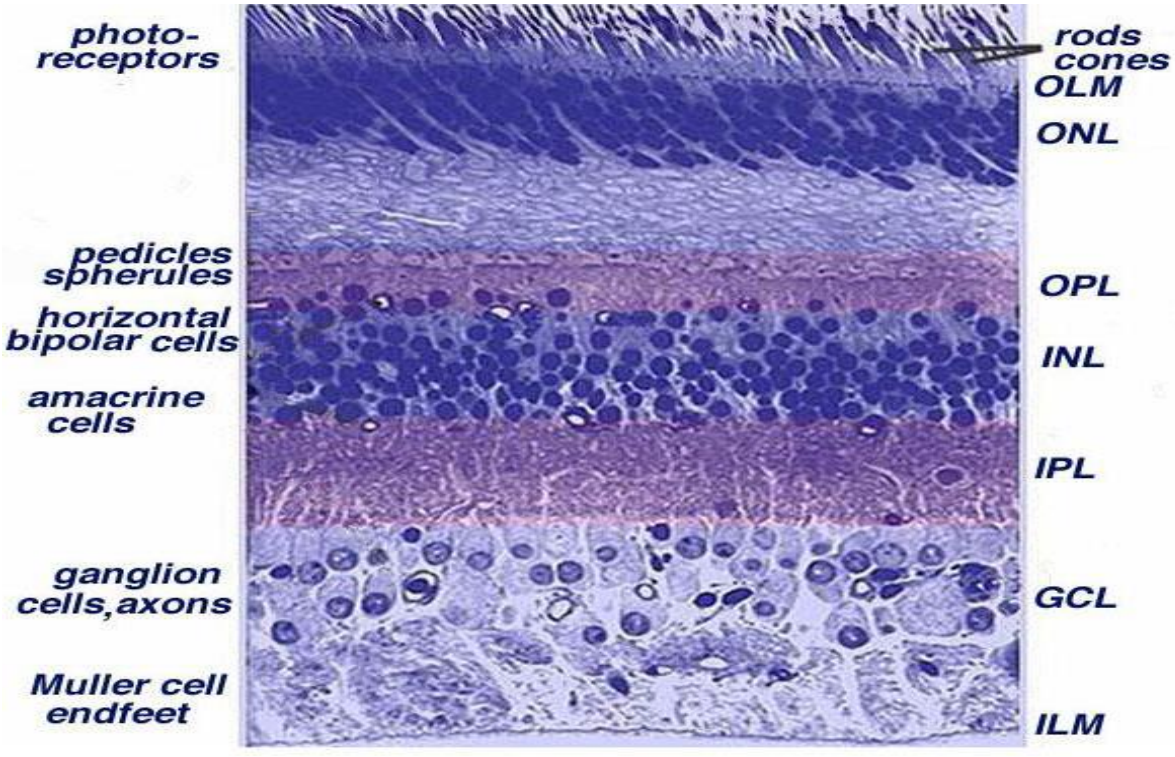


Figure 5.2: Light microscopic appearance of human retina

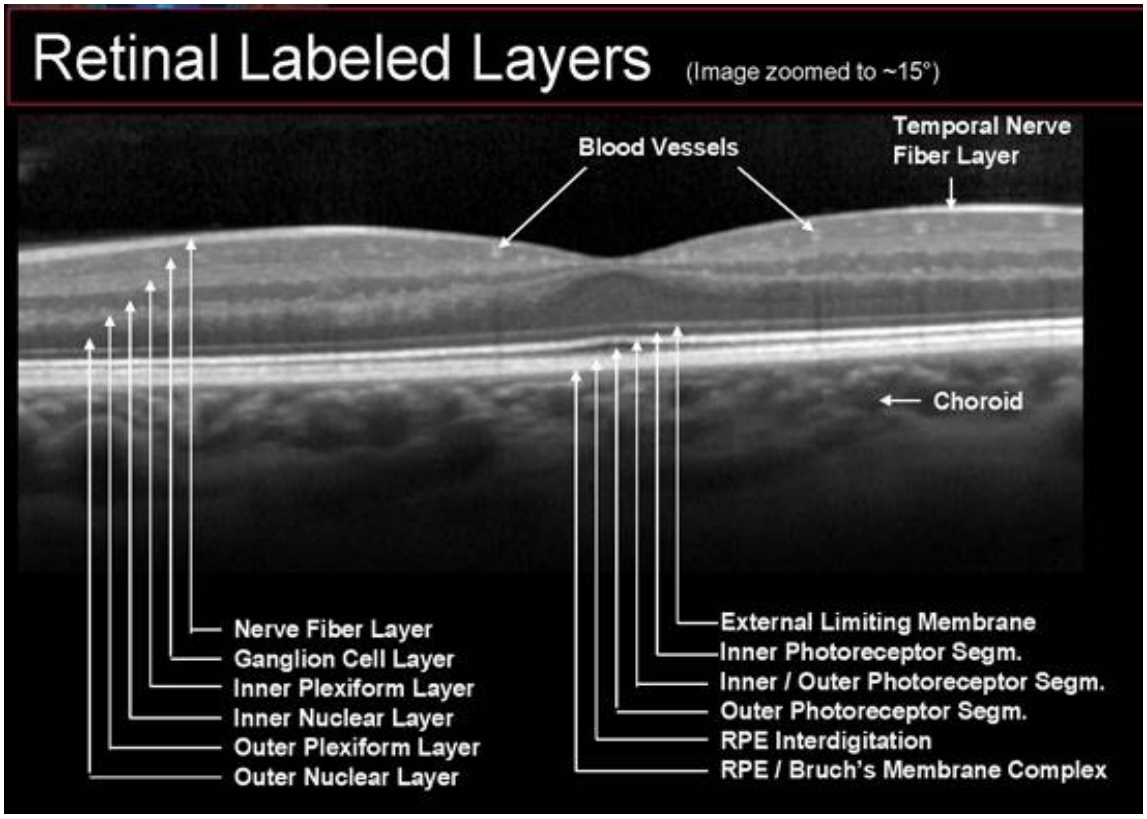


Figure 5.3: Layers of retina and choroid on OCT (Heidelberg)

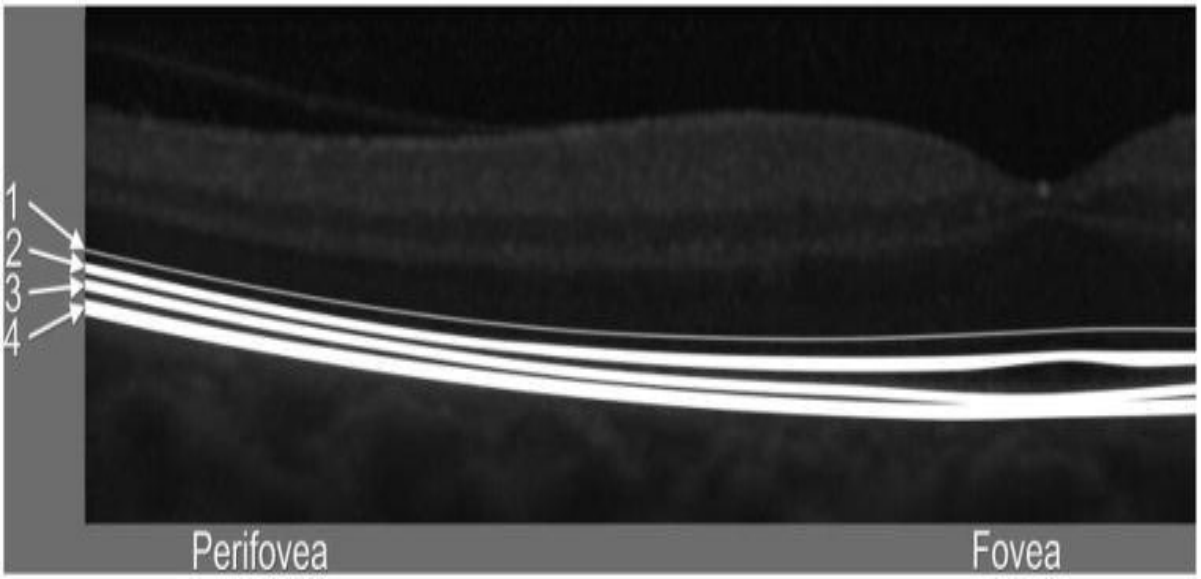


Figure 5.4: Close inspection reveals four reflective bands on OCT
1=external limiting membrane, 2=Ellipsoid layer, 3=Inter-digitation zone, 4=Retinal pigment epithelium (Heidelberg)

1) The external limiting membrane band (ELM) is located at the boundary between the cell bodies (nuclei) and the inner segments of the photoreceptors, and comprises clusters of junctional complexes between the Muller cells and the photoreceptors.

2) The ellipsoid zone (EZ), which was previously referred as the photoreceptor inner segment/outer segment (IS/OS) junction, formed mainly by mitochondria within the ellipsoid layer of the outer portion of the inner segments of the photoreceptors. In a normal fovea, the distance from the EZ line to the ELM is shorter than that from the EZ line to the RPE.

3) The interdigitation zone (IZ) corresponds to the contact cylinder represented by the apices of the RPE cells that encase part of the cone outer segments. This layer was previously referred to as cone outer segment tips (COST) or rod outer segment tips (ROST), and it is not always distinguishable from the underlying RPE layer, even in normal subjects. Although this band is called Verhoeff membrane by Verhoeff as an anatomical structure girdling RPE cells, now known as junctional complexes between RPE cells.

4) The retinal pigment epithelial band is formed by the RPE and Bruch's membrane (indistinguishable from each other in a normal state using current SD-OCT systems). In the fovea, this band is thicker, which indicates that choroidal structures may also contribute to the hyper-reflectivity of the RPE band at this location.

5.6. OCT of the Choroid

Enhanced Depth Imaging (EDI)-OCT :Imaging beyond the RPE

- EDI-OCT is a new imaging modality to enable high-resolution imaging of the choroid and lamina cribrosa.
- It is noninvasive, reproducible and comfortable to patients as standard OCT.

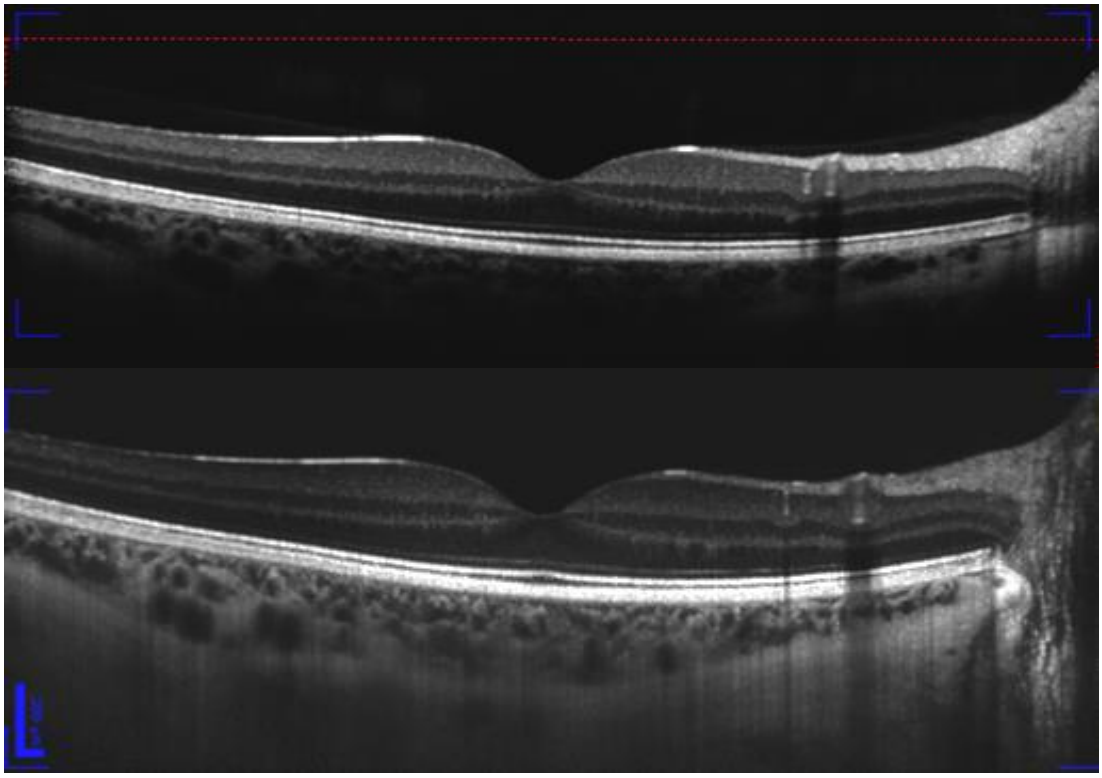
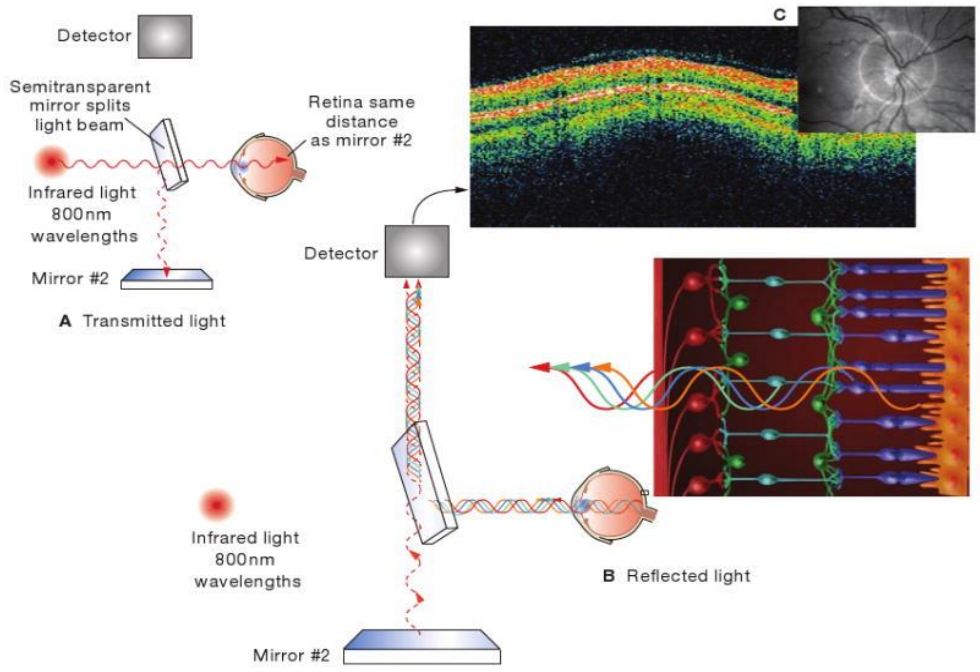


Figure 5.5: Enhanced depth imaging showing clear definition of choroid (below) compared to poor definition of choroid (above)

Figure 5.6: Principle of EDI OCT (Heidelberg, Germany)



Chapter 6

Functional assessment of visual system

6.1. Function of visual system

Introduction

Visual dysfunction is one of the most common clinical manifestations of multiple sclerosis (MS). MS clinical trials did not include visual outcomes a decade ago. Snellen's visual acuity chart was the common visual testing at that time and the lack of change of vision parameters with concurrent change in EDSS might be one of the justifiable reasons. The Optic neuritis treatment trial (ONTT) [96, 97] set the scene for the current trend in assessment of visual pathway.

6.2. Senses of the visual system

The stimulation of retina results in four types of sensation -light sense, form sense, colour and contrast sense.

a) Form Sense

Form sense is a faculty that enables us to perceive the shapes of the objects. The ability to perceive this is called visual acuity as termed by Donders in 1861. Visual acuity includes sharpness of retinal focus and ability of interpretive focus in the brain. The sharpness of focus is mainly dictated by the function of the cones in the fovea. The brain has interpretive components such as recognition of objects, resolution of 2 spatially separated targets and alignment.

Resolution is the ability to resolve two spatial separated target points. It normally subtends a visual angle of 1 degree at the nodal point. This equates to 0.0004mm size of the object which is the size of the foveal cones[98]. This is ordinary visual acuity; a function of the cones. The ability to resolve 2 line segments is termed as hyperacuity or vernier acuity. The visual angle is much smaller than the 1 degree and the processing most probably takes place in the visual cortex.

b) Contrast sense

Contrast sensitivity is a measure of the ability to discern between luminance of different levels in a static image. Contrast sensitivity varies between individuals, reaching a maximum at approximately 20 years of age, and at spatial frequencies of about 2–5 cycles per degree. In-addition it can decline with age and also due to other factors such as cataracts, glaucoma, optic nerve disease and diabetic retinopathy[98.]

c) Colour sense

It is the ability to distinguish between colour and colour tones. There are three primary colours, red, green and blue. Colour is best appreciated in bright light whereas they appear grey in dark, a phenomenon called Purkinje's shift. Perception of colour begins in the cones, which contains pigments three different spectral sensitivity. This results in tri-chromatic colour vision. The

Opsins (photo pigments) present in the L and M cones are encoded on the X chromosome; defective encoding of these leads to the two most common forms of colour blindness[100].The OPN1LW gene, which codes for the Opsin present in the L cones, is highly polymorphic (a recent study by Verrelli and Tishkoff found 85 variants in a sample of 236 men)[101]. A very small percentage of women may have an extra type of colour receptor because they have different alleles for the gene for the L opsin on each X chromosome. X chromosome inactivation means that only one Opsin is expressed in each cone cell, and some women may therefore show a degree of tetra-chromatic colour vision.[102] Variations in OPN1MW, which codes the opsin expressed in M cones, appear to be rare, and the observed variants have no effect on spectral sensitivity.

6.3. Cone cells in the human eye[103,104]

Name	Cone type	Range	Peak
S	B	400–500 nm	420–440 nm
M	Γ	450–630 nm	534–555 nm
L	P	500–700 nm	564–580 nm

6.4.Theories of color vision

Tri-chromatic theory of colour vision

Thomas Young in 1802 and thereafter Hermann Von Helmholtz in 1852 proposed that all human vision occurred through the combination of sensitivity to red, green, and blue. This theory came to be known as the Young-Helmholtz trichromatic theory of colour vision. The finding that there are three types of colour-sensitive cone receptors in the retina supported the three-colour theory[105]. One set of receptors is sensitive to long wavelengths such as red, one to medium wavelengths such as green, and one is sensitive to short wavelengths such as blue. The basic idea was that the eye responded to three primary colours, and combining the three primary colours of additive colour mixing formed all the other colours.

Opponent Processing Theory of Colour Vision

As the trichromatic theory did not explain it all, Ewald Herring, the father of the opponent processes theory came up with some interesting thoughts. Firstly, he noted that there are certain pairs of colours one never sees together at the same place and at the same time such as one does not see reddish greens or yellowish blues. Secondly, he noted a distinct pattern of colour of the after images we see; for example if one looks at a unique red patch for about a minute and then

switches the gaze to a homogeneous white area they will see a greenish patch in the white area.

Herring hypothesized that tri-chromatic signals from the cones fed into subsequent neural stages and exhibited two major opponent classes of processing.

1. Spectrally opponent processes, which were red vs. green and yellow vs. blue.
2. Spectrally non-opponent processes, which were black vs. white. This opponent process model lay relatively dormant for many years until a pair of visual scientists working at Eastman Kodak at the time, conceived of a method for quantitatively measuring the opponent processes responses. Leo Hurvich and Dorothea Jameson invented the hue cancellation method to psychophysically evaluate the opponent processing nature of colour vision.

Due in large measure to the efforts of Hurvich and Jameson the opponent processes theory attained a central position shared with the trichromatic theory. Consequently, with the quantitative data provided by the psychophysics and direct neurophysiological responses provided by electrophysiology opponent processing is no longer questioned. Based on the existence of colour afterimages, Ewald Herring proposed the opponent process theory[106] of colour

vision in 1878. He suggested that colour vision occurred in three channels where "opposite" colours (called complementary colours) are in a form of competition. For example, red and green are complementary colours. When you stare at something red, your redness detectors are worn out or fatigued. Their opponents, the green receptors, gain the upper hand, and you see a green after-image after staring at a red dot.

6.5. Men and colour blindness:

Colour-blind people usually have a complete (achromatopsia) or incomplete absence (dyschromatopsia) of colour. There might be one or more missing cone types: red-sensitive, green-sensitive, or blue sensitive. The result is a disorder in one or both colour channels.

The most common type of colour blindness is red/green colour blindness. Genetic studies show this type of colour blindness is usually caused by a defective gene on the X chromosome. If this gene is defective, women (having two X chromosomes) are "protected" by a duplicate copy of the gene on the other X chromosome. Males (having one X and one Y chromosome) do not have the extra copy, so red/green colour blindness is about 20 times more common in men than in women

6.6. Monochromat

A person with no colour sensitive pigments, therefore no colour vision, is called a monochromat (one-color person). To such a person, the world looks like a black-and-white TV picture. Colours are shades of grey.

6.7. Dichromat

A person with a defect in one channel-either the red/green or yellow/blue channel-is called a dichromat. Both colours in a channel are affected, so if the person cannot distinguish red that same person cannot distinguish green. A person who cannot see blue as a distinct colour will also not see yellow as a distinct colour.

6.8. Tri-chromat

People with normal colour vision use all three channels (black/ white, red/green, and yellow/blue) and are called tri-chromats.

6.9. Testing of visual sense in Multiple Sclerosis

6.10. Form and contrast sense

Vision is commonly tested using Snellen's charts. Ophthalmologist, Herman Snellen in 1862, developed the chart. The common Snellen chart is printed with eleven lines of block letters. The size of the letter drops from being large in the

first line and being small in the very last line. The principle behind the sizing of the letter is based on the visual angle it subtends on the fovea, whole letter subtends an angle of 5 degree and each segment in the[107] letter subtends an angle of 1 degree at the specified viewing distance.

Normally the test is taken at a distance of 6meters or 20 feet away. The smallest row that a person can read is the actual visual acuity of the patient. The test can further be tested with pinhole in front of the eye to rule out media opacity and refractive error. The letters are designed opto-types and comply with simple geometry. The thickness of the lines equals the thickness of the white spaces between lines and the thickness of the gap in the letter "C". The height and width of the optotype (letter) is five times the thickness of the line. Only the ten Sloan letters C, D, E, F, L, N, O, P, T, Z are used in the common Snellen's chart.

Visual acuity = Distance at which test is made / distance at which the smallest optotype identified subtends an angle of 5 arc minutes.

Patients unable to read the chart at 6 m are asked to read it at 3m and 1m. For patients unable to read the letters on the closer placed charts are asked to count fingers close to face.

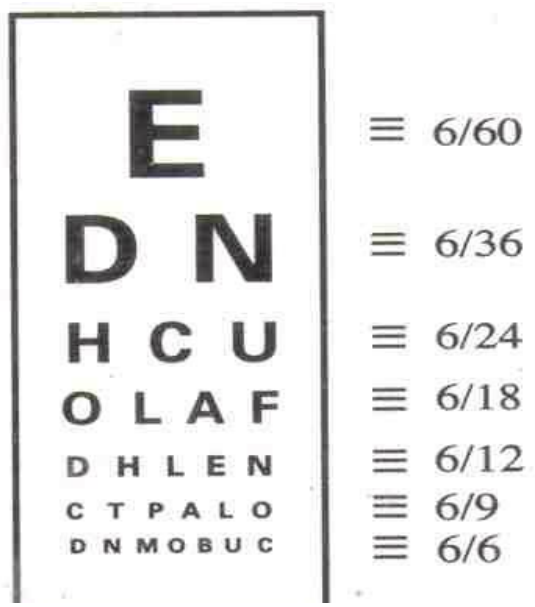


Figure 6.1: Snellen's charts

Limitations of Snellen's chart:

* Tests only form sense with no indication of contrast, colour, depth perception or field of vision.

* Crowding phenomenon

* Forced choice option

* Does not measure quality of vision

* Overall a poor measure of vision

6.11. Introduction of Contrast charts (LogMAR) in Multiple sclerosis

In the late 1990's, the National Multiple Sclerosis Society Clinical Outcomes Assessment Task Force developed the MS Functional Composite (MSFC) in response to the need for more sensitive neurological outcome scale[108, 109]. Snellen's high-contrast visual acuity (VA) did not change over time or demonstrate concurrent changes with EDSS scores. Contrast sensitivity, as tested by line gratings and letter charts in MS and by Pelli-Robson charts in the Optic Neuritis Treatment Trial (ONTT), had been shown to be a sensitive measure of afferent visual function, even among patient with Snellen's acuities of 20/20 or better[110.] Importantly, measures of low-contrast vision are predictive of "real-world" visual tasks such as reading rate, facial recognition, and driving .Hence was the introduction of Sloan's contrast charts to the clinical scenario in MS.

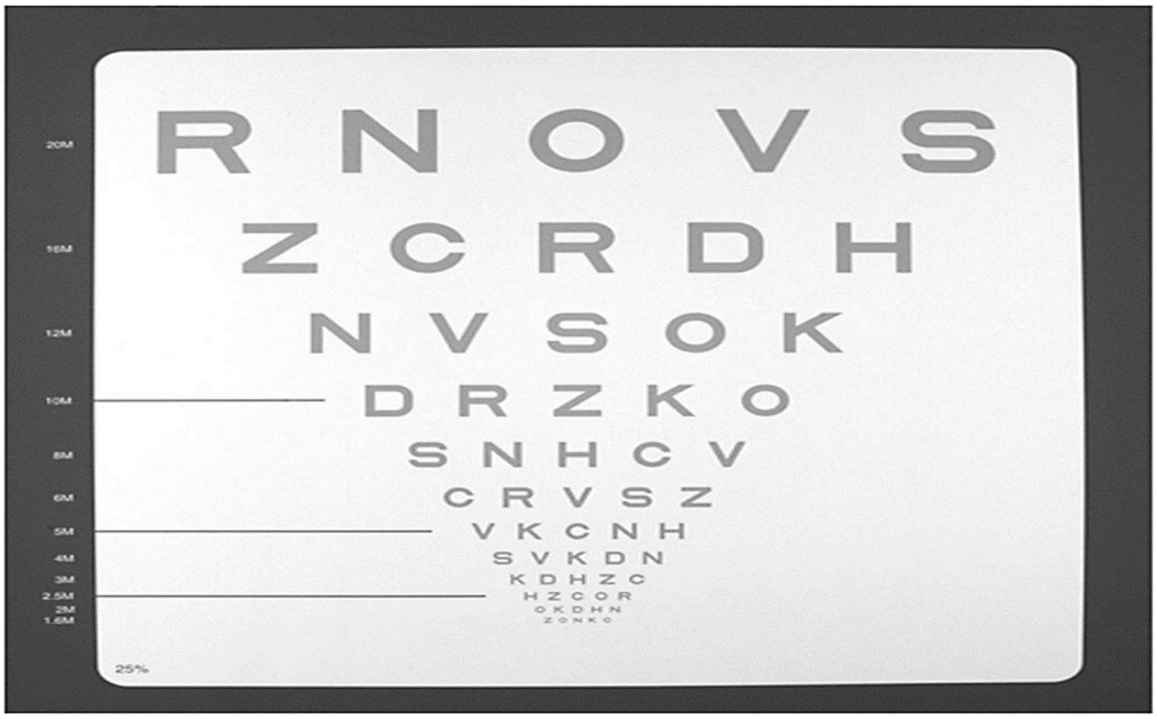
6.11a.Logarithmic Sloan contrast charts

The charts of the low-contrast (gray letters on white) charts are “cousin” of the ETDRS (early treatment of diabetic retinopathy study) high-contrast VA charts used in ophthalmology clinical trials (Figure 6.2). Sloan charts have a standardized format based on the ETDRS VA charts. Three contrast levels have been used in MS trials and research studies, including 100% (high-contrast, used to measure VA as a descriptor of the study cohorts), 2.5%, and 1.25% (lightest contrast level). Charts are scored letter by letter, and numbers of letters identified correctly constitute the score for each chart.

Figure 6.2: LogMAR chart

Most recently, visual improvement and loss by the low-contrast acuity chart has been defined as a 7-letter change in score, while 5-letter changes in high-contrast VA are now considered clinically significant for patients with good visual acuity[111]. This threshold represents a change that exceeds that which would be expected from repeated testing when there was no real change, and to correlate with RNFL axonal loss in patients with MS.[112]

Figure 6.3: Sloan chart 2.5%



Illumination is a very crucial part in contrast vision testing. Without a constant and standardized illumination, contrast charts may be inaccurate. Precision Vision Contrast Charts are recommended with the use of our retro illumination

cabinets. With this illumination, contrast levels are more accurate and testing is more reliable. Controlled illumination is crucial for achieving accurate contrast sensitivity results.

6.12. Colour vision testing in Multiple sclerosis

The Ishihara Pseudo isochromatic charts have in countless times in the past tested colour vision. With the invent of the sensitive Anomaloscope , the Ishihara chart has been known to be a vague indication of colour deficiencies. One of the pseudo isochromatic charts which over performs the anomaloscope is the Richmond HRR chart, 4th edition.

Sixty years ago LeGrand Hardy, Gertrude Rand, and M. Catherine Rittler commenced development of a colour vision test that later was made commercially available under the name “HRR Pseudoisochromatic Plates” (Hardy et al., 1954) .Based on a fail criterion of three errors on the transformation and vanishing plates, the sensitivity of the Ishihara test is around 99% and the specificity is 94%the sensitivity of the Richmond HRR test has been reported to be 100% sensitive and the specificity to be 96%.

Abnormalities in colour vision are common in patients with multiple sclerosis (MS), especially after optic neuritis (ON),even though acquired dyschromatopsia is also well documented in MS patients[113] without ON.

Colour deficits can be detected in 70% of MS patients without a history of previous ON by using the Farnsworth-Munsell (FM)-100 tests.

Although the mechanisms involved in colour vision impairment are not completely understood[114], the result of functional tests of colour vision suggests that impaired colour vision is produced primarily by injury to the anterior visual pathway[115] rather than to postchiasmatic or posterior visual pathway structures.

6.13. Testing of colour on HRR charts

The test chart is held on a rack of illuminator about 30 inches from the patients. Any source of extraneous light should be turned down to avoid false interpretations. The test charts have 24 plates in total. 1-4 are test charts, 5-10 are screening charts and 11-24 are testing charts .See test reporting chart below. .Six plates (screening series) present the most difficult yellow, blue, red, and green colours and separate normal from defectives. Subsequent 14 plates diagnose as to the extent (mild, medium or strong) and type of defect (protan, deutan, tritan).

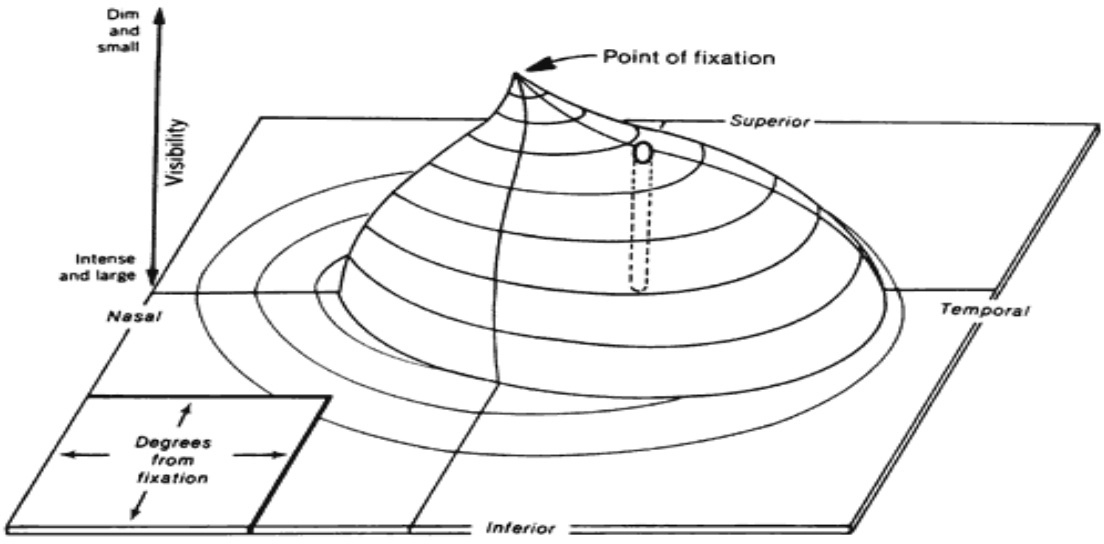
Figure 6.4: HRR chart

6.14.

Field of vision

The VF is a three dimensional cone (Traquair's island of vision), with its apex at the nodal point of the eye, and its base at infinity (or at whatever distance we plot it by a perimeter screen or bowl) (Figure 6.5). The purpose of visual field testing is to define the topography of the island of vision to recognize any variation from normal.

Figure 6.5: Hill of vision



The plotted VF (the base of the cone) extends for approximately 60 degrees superior, inferior, and nasal and 100 degrees temporally. For practical purposes, the VF plot may be divided into three major parts: the central 30 degrees, the peripheral field (from 30 to 60 degrees), and the temporal crescent (Figure 6.6).

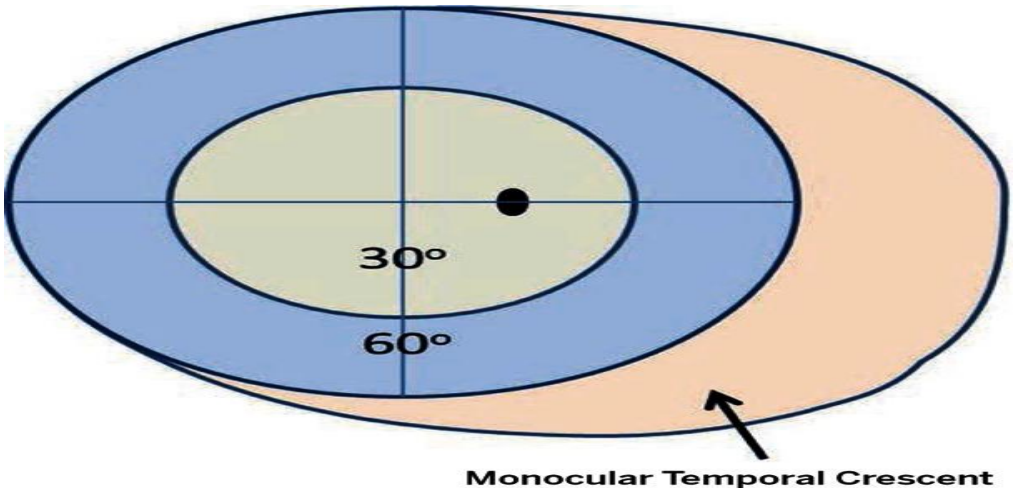


Figure 6.6: Field of temporal vision

The visual field test is designed to assess the sensitivity of the eye as well as the extent of the peripheral vision.

The patients chin is placed on a chin-rest to start a series of flashing lights on a bowl-shaped screen. The lights may vary in brightness and will appear in different positions in your field of view. Typically a 24-2 SITA test takes about 5 minutes per eye and a 30-2 about 7 minutes per eye. Full threshold tests can take up to twice as long.

6.15. Humphrey SITA and Full Threshold Testing

The Humphrey Visual Field is standard tests that allow us to look for defects in the sensitivity of the eye.

Threshold Visual Fields:

Threshold testing of visual fields identifies the limit of the sensitivity of the eye at programmed locations in the visual field.
The most common Humphrey test is the 24-2, which tests the central 24 degrees of vision. The Humphrey 10-2 test permits monitoring of patients with very restricted fields and the 30-2 has more peripheral points. The following strategies are available for the 24-2, 10-2, 30-2 tests and 50-2

Full Threshold - makes the least assumptions about the patient's vision. The brightness of the stimulus is varied at each location in order to find the threshold value. The results are based on the last point seen at each location, so patient fatigue may affect the accuracy.

SITA Standard (Swedish Interactive Threshold Algorithm). With the benefit of research into visual fields, the SITA test is able to make predictions of threshold values by analysing the patient's previous responses. By analysing the data in this way the length of the test is reduced, often less than half the length of a

similar full-threshold test. The results are calculated using all of the data collected, so errors may be identified and corrected automatically.

SITA Fast - the fastest threshold test. Best used with reliable subjects.

Stimulus Size - Full threshold tests may be carried out using different stimulus sizes. These equate to the stimulus sizes used by the Goldmann Perimeter. Humphrey tests are normally carried out with stimulus size III, but patients with poor vision may be tested with a size V stimulus. Please note the SITA tests are only possible with a size III stimulus.

Stimulus Colour - the Humphrey test is normally carried out using a white stimulus against a white background. However, the stimulus colour may be changed when monitoring colour dependent changes.

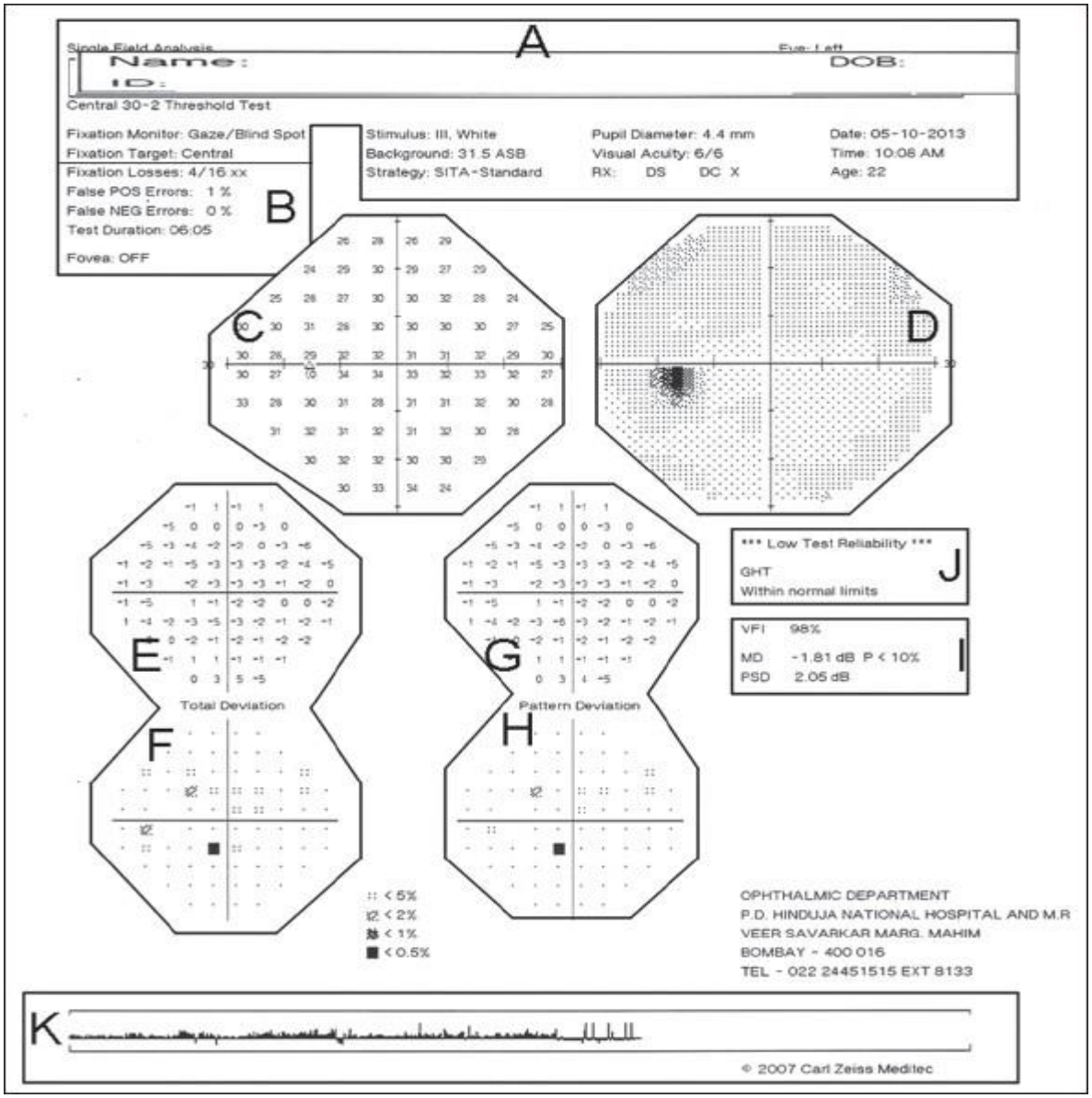
Progressor Visual Field Analysis

Using the Institute of Ophthalmology's Progressor software, we are able to monitor progression of visual fields using linear regression analysis. The software is able to identify progressing points from a series of Humphrey 24-2 Full Threshold or SITA examinations and marks them according to clinical significance.

6.16. The Humphrey printout

The test results (with or without the statistical analysis) are usually printed on one sheet called the printout (Figure 6.7). When examining the test results, the two printouts (right eye print out in your right hand facing your right eye, and left eye printout in your left hand facing your left eye), and examination is done together.

Figure 6.7: Humphrey's visual field print out



6.17. Patient’s data and test parameters (A& B on Fig 6.7)

These are at the top of the printout.

- The first priority is to make sure that those fields belong to the patient in question, and that the specific program requested has been used.
- The date of birth has to be correct (for comparison with range of normal values for age). A pupil diameter of at least 2.5 mm is essential to avoid overall depression of test values. Finally, using near correction lenses, and/or high astigmatic error correction lenses is strongly advisable to help the patient appreciation of the test targets. A frameless lens may be best suited for that, but other lenses could be used and allowance for any rim artefacts made during interpretation.

Reliability indices

The second priority is to check the patient' performance (reliability). These parameters are printed at the top left hand side of the print out in the Humphrey perimeter.

Fixation loss: Normally this is between 0 to 2%. If the loss exceeds 20%, this is generally considered as poor reliability. However, it may also be an indicator of advanced glaucoma with an abnormally large blind spot. Other indicators of advanced glaucoma should be looked up first, before disqualifying the test results as unreliable based on poor fixation.

- False-positive or false-negative responses. Scores in excess of 20-30% indicate a test of questionable reliability

The grey scale (D on figure 6.7)

- This is a graphic representation of the recorded threshold sensitivities in the numeric scale. Regions of decreased sensitivities are displayed in darker tones. The grey scale plot is very useful for displaying patterns of loss (nerve fibre layer defects versus neurological defects) and thus should be inspected first. When looking for such patterns, both right and left grey scales should be inspected together. If any visual field defect related to pathology of the visual pathway distal to the lamina cribrosa is revealed, then attention is directed to other tests to examine the rest of the visual pathway.
- It is also a very useful tool in explaining – to the patient and family – the stage of the disease and its progress over time.

The numeric scale(C on figure 6.7)

This is the main test result. It shows the retinal sensitivities at the different test locations, expressed in Decibels (dB).

The numbers expressed in the numeric scale may be looked at as heights, and with the higher values in the centre, and the least values at the periphery, one can ‘see’ the centre of the hill of vision in a three dimensional way.

6.18. Statistical analysis of test results

Inclusion of statistical analysis software is the reason for the widespread popularity of static perimetry. Computers can store a huge amount of numbers and use them to look for patterns, compare them with stored database, and perform all kinds of analysis on them.

The Stat-PAC software is a commonly used analysis tool in both Humphrey. It is used to look at suspicious clusters of numbers, analyse them, and monitor their change over time.

Total deviation plot (E&F on figure 6.7)

- These numbers show the difference (in dB) between the test results and the normal values expected for the patient's age group. 0 dB = no difference (normal), while −13 dB = large depression from normal value (see the total deviation plot in Figure 6.7). The true value of the defect in this instance should be −17 dB, but the computer allows for a variability of −4 dB.

- This is in fact a plot of the probability of each point change being normal. If less than 0.5% (indicated by the solid black squares), then the point change is highly unlikely to be normal.

Pattern deviation plot (G& H on figure 6.7)

This particular analysis tool is helpful for detecting visual field defects (scotomata) in the presence of media opacities, such as cataract. It does this by looking at the overall sensitivity changes in the hill of vision (the 'pattern' here is the conical shape of the hill of vision). If there is an overall depression (all test values are reduced from normal due to cataract), then it will subtract this value from all test points, leaving behind clustered field loss (localised defects), which may be due to glaucoma.

'Inclusion of statistical analysis software is the reason for the widespread popularity of static perimetry'

Global indices (J&I on figure 6.7)

These numbers represent mathematical summaries of all the sensitivity values produced by the test. They are useful tools for having a quick idea about the entire field, and sequentially compare test results for the same eye (change analysis). Looking at them however, does not replace examining other test data in detail. Note that the newer SITA (Swedish Interactive Threshold Algorithm) software has just a pattern standard deviation.

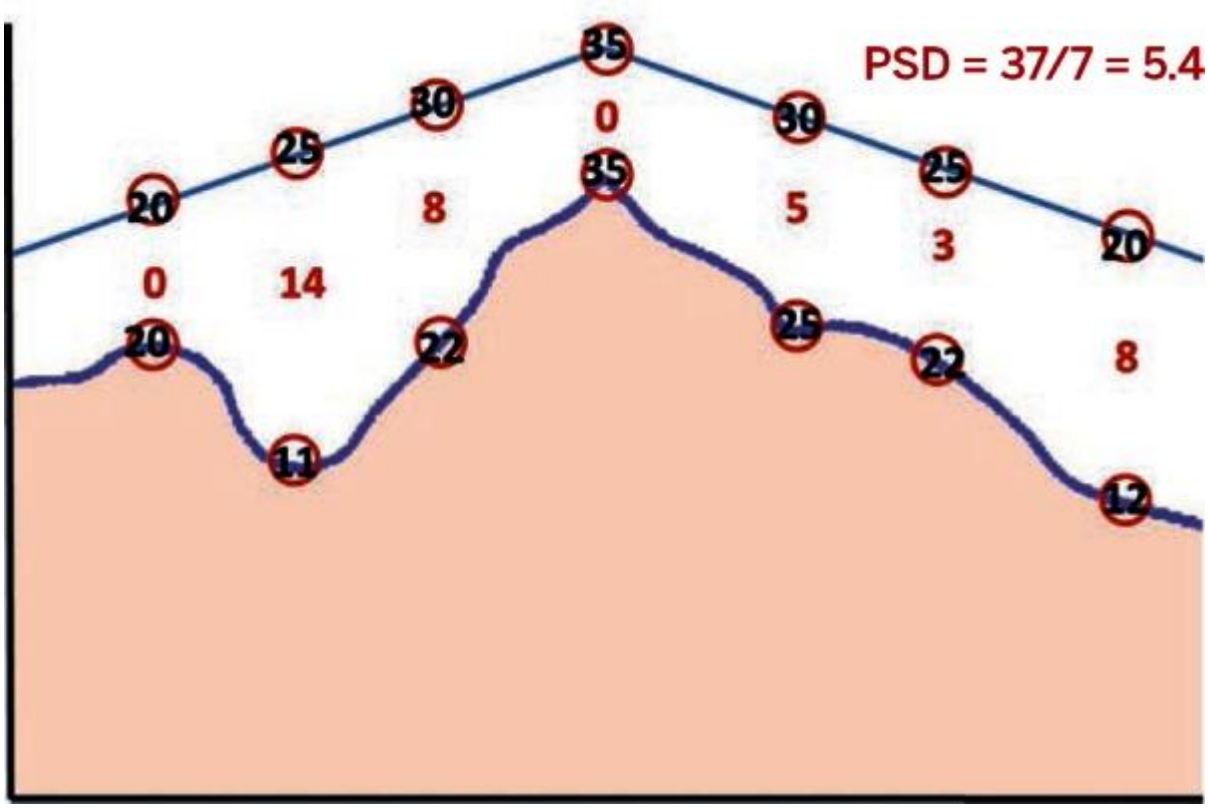
Mean deviation

This number reflects the overall depression (deviation from normal values) of the field. All the obtained values of the test are added, and divided on the number of test locations. This gives the mean value of the test. The same is done for the normal expected values stored in the computer database. The difference between the 2 values represents the MD. Normally it should not exceed −2 dB. If the MD is significantly outside the normal, then a P value is assigned to it.

Pattern standard deviation

For practical purposes, the pattern standard deviation (PSD) reflects the degree of departure (difference) of the measured VF pattern (shape) from the normal hill of vision. A small PSD reflects a smooth uniform hill of vision (Figure 6.9), while a large PSD value reflects an irregular hill of vision (Figure 6.8).

Figure 6.8: Hill of vision on Pattern standard deviation (steep)



The pattern standard deviation (PSD) is the difference between the expected data (the straight line at the top) and the patient's own data (the irregular line beneath it). Here, the sum of the differences is 38, which, when divided by number of test locations, yields 5.4. This reflects an irregular hill of vision.

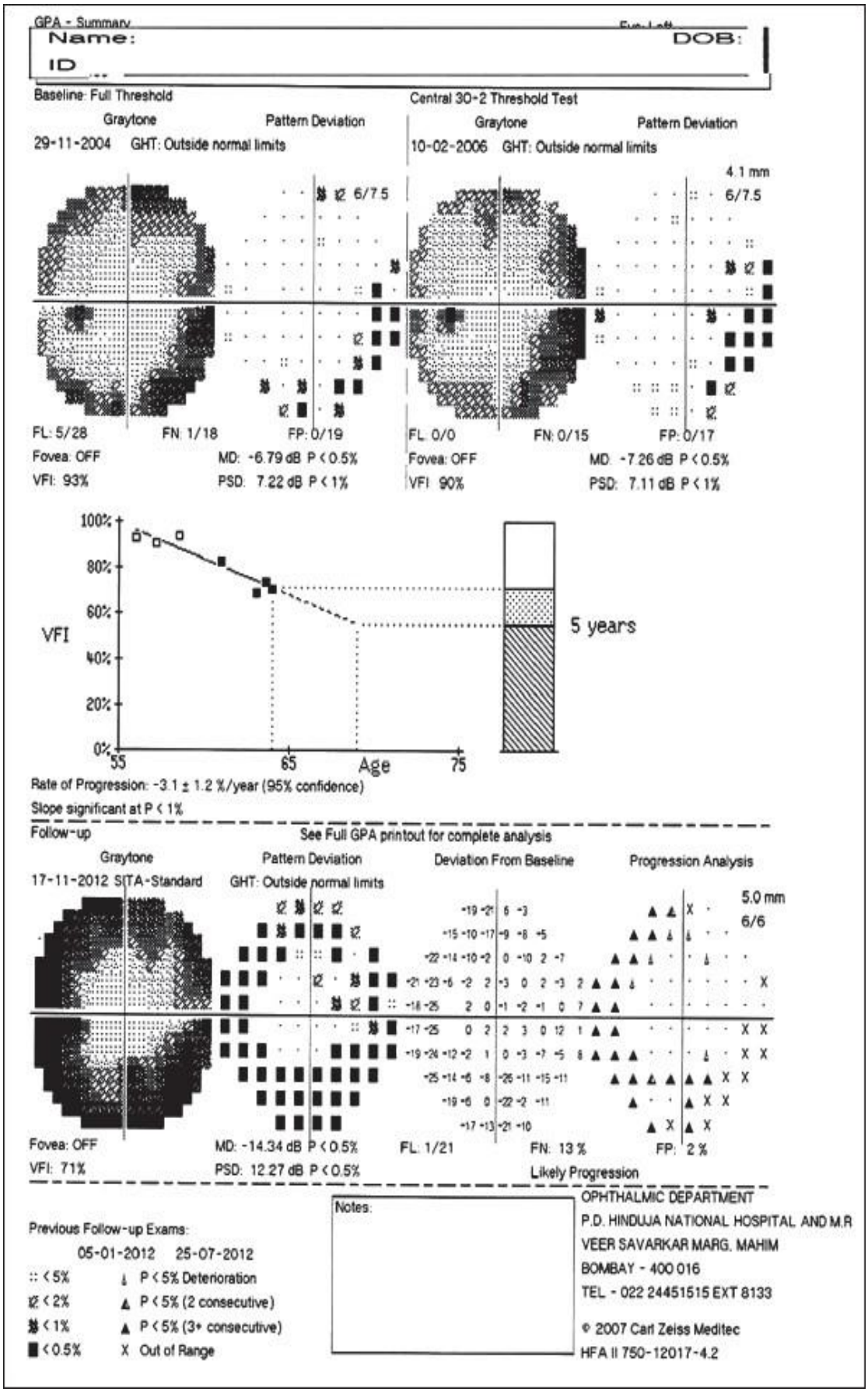
Figure 6.9: Hill of vision on PSD (smooth)

The sum of the differences is here is 1.7, a much smaller number, which reflects a much smoother hill of vision with a more normal pattern to the visual field.

6.19 The change analysis printout

This is a simple graphic representation of summaries of sequential VF tests for the same eye using all global indices. It helps to evaluate progression (or stability). Care should be taken when using change analysis, because it requires many reliable fields (at least 4 excluding the first one to avoid learning mistakes). To compare results from a small number of fields, one can use the 'Multiple field's printout', to visually track changes between the tests in question. The change analysis printout allows us to track changes over time.

Figure 6.10: Progressive field analysis



The hemi-field analysis

This software allows comparison of VF defects across the horizontal axis (looking for – and comparing – nasal steps). As such it alerts you to the need to re-examine the printed results, looking for such differences. The three important responses to look for are:

1. Within normal limits (no differences)
2. Borderline (early differences)
3. Outside normal limits (obvious differences between the upper and lower halves of the field)

Chapter 7

Methodology

Chapter 7: Methodology

Introduction

This project aims to introduce a number of advancements in the development of visual function outcomes in MS as well as OCT as a novel technology that enables objective analysis of the processes of neurodegeneration. In addition, we present representative group data from visual function, OCT measures, and visual field indices in patients with MS, ON, and disease-free control (Table 1).

These data may be used to provide initial reference values and should be viewed in the context of 1) the continually-evolving field of vision in MS; 2) the potential challenge inherent in applying group data to individual patients; and 3) the perspective that even the small observed differences in mean OCT values have been shown to correlate with clinically meaningful changes in visual function in MS patients.

“Eye is a window to the brain”

Structural loss of neurons in the optic nerve/retina in MS (as measured by OCT) is related to loss of vision on functional visual tests and also to neuronal loss as measured by EDSS.

Primary Outcome:

a) To study the anterior visual pathway with a view to correlate structure with function.

Secondary Outcome:

b) To correlate the structure of anterior visual pathway with neurological function (EDSS, QOL)

Overall this may throw light on visual outcomes as a marker for early diagnosis, disease progression and monitoring the response in patients treated with disease modifying therapy (DMT).

Use of visual parameters in diagnosing and monitoring the disease in Multiple sclerosis is not a common practice in the United Kingdom or elsewhere.

Primary Objectives:

1) To study the sensitivity and specificity of non-invasive vision tests such as LogMAR, colour charts and contrast sensitivity charts in diagnosis and monitoring treatment in relapsing remitting multiple sclerosis. Snellen's visual acuity is a routine test in assessing vision in MS patients. It will be compared with specific visual charts such as LogMAR, HRR and contrast charts.
2) To perform non-invasive eye investigations such as OCT of retina, choroid and optic nerve (Optical coherence Tomography)) to detect nerve fiber loss in the retina and optic nerve head in MS.

Secondary Objectives:

1) To find a correlation between visual outcome measures on OCT with disability outcome measures such as EDSS (Expanded disability status score) and QOL in Multiple Sclerosis (MS).
2) To correlate point sensitivity on visual field with thickness of nerve fiber on posterior pole OCT
3) To compare the structural and functional visual changes in patients with and without optic neuritis.

4) To explore if age of onset of optic neuritis correlates with visual outcome
5) To evaluate the usefulness of EDI-OCT in studying the choroid.

Methodology

The project “Structure and function of the visual pathway in demyelinating optic neuropathy " was carried out in the University Hospital of North Staffordshire, now called the Royal Stoke University Hospital.

The project was approved by the IRAS and peer review committee at Keele University.

The study integrated work between the department of Ophthalmology and Neurology. Senior consultants in Ophthalmology and Neurology along with the senior Orthoptist monitored the activity and patient turn over.

A total of 60 patients (120 eyes) and 25 controls (50 eyes) were included in this prospective case control study. Patients attending their routine Neurology outpatient clinic were identified 4 weeks in advance on the patient record system of the UHNS trust. Phone calls were made to discuss the project with the patient. Patient information leaflet and consent form were posted to those keen on being enrolled in the study.

On the day of the visit, patients were given time for discussion, questions and answers before proceeding to the actual tests. Most patients were happy to contribute to this research with only one patient not happy to be recruited as he lived very far away. The support from the Ophthalmology department to access the various investigative modalities was excellent which made carrying the project more enjoyable.

Further, weekly meetings were held between the Prof Hawkins (PhD supervisor), Mr Jones and Mr Ragheb (Ophthalmology) and Prof Strange / Dr Ramachandran (Interest in MS and statistics) along with quarterly meetings with Prof Jones (Statistician).

Study population

Cases:

Patients with Relapsing remitting type of MS (RRMS) who fulfilled diagnostic McDonald criteria 2010 were included in the study. CIS (clinical isolated syndrome) and suspects were not included in the study due to lack of diagnostic criteria for this group. Patients with or without optic neuritis and those on DMT treatment were included.

Controls: Normal volunteers

Inclusion Criteria

- Age: 18- 65 and able to consent
- Diagnosis of Relapsing Remitting MS using Mc Donald's criteria (2010) in patients with or without treatment, with or without optic neuritis.

Exclusion criteria

- Any progressive neurological disorder (other than MS),
- Any medical condition or limiting psychiatric disease (including depression, bipolar and psychosis) that may interfere with the subject's ability to cooperate and comply with study procedures
- Any state of immunosuppression different from MS;

- Any ophthalmological causes for retinal damage different from MS or major difficulties for OCT evaluation such as severe refractive defects [myopia > −6.0D or axial eye length >26 mm; hypermetropia > 5 D; cylinder > 3D], optic disc drusen, cataract and glaucoma.
- Current or previous treatment with a drug involved in toxic neuropathy, such as Ethambutol, Isoniazid, Linezolid, Gentamycin, Chloramphenicol, Vincristine, Penicillamine.
- Recent history of acute optic neuritis (<6 months). Patients with ON within 6 months of the acute episode were not eligible.
- Previous diagnosis of Diabetes Mellitus or impaired fasting glucose (≥126 mg/dl or ≥ 200 mg/dl after oral glucose tolerance test)
- Inability to undergo MRI: reduced renal clearance (screening: GFR < 45 ml/min), history of severe hypersensitivity to gadolinium-DTPA, claustrophobia
- History of substance abuse in the last 5 years including alcoholism (>40 g/day for women and 60 g/day for men) and severe tobacco use (>20 cigarettes/day). Patients with acute relapses or systemic steroid treatment in the previous month can be included 2 months after the acute episode
- Unable to position on the various testing equipment
- Unable to consent

On the day patients identified and happy to be recruited were invited for discussion about the visual project. There was a ten-minute session for question and answers, which included history taking and consent process.

Patients were invited to read the test charts, which included

1) The 100% LogMAR contrast test chart

2) The Sloan 2.5% and 1.25% contrast test chart

3) HRR colour test chart, followed by

4) Humphrey's visual field test

Tests 1-4 were done with appropriate corrective glasses if required. Further tests were in the Ophthalmic imaging rooms where patients had

5) OCT tests to study

a) Macula (Fig 7.1)
b) Optic disc
c) Posterior pole (Fig 7.2)
d) Choroid.

All these tests were done in between the normal NHS workload. UHNM ophthalmic staff offered great deal of support during the recruitment process.

Controls outside the study group were tested for coefficient of variability prior to the start of the recruitment.

Fig 7.1: Image of the macula and correlating OCT.

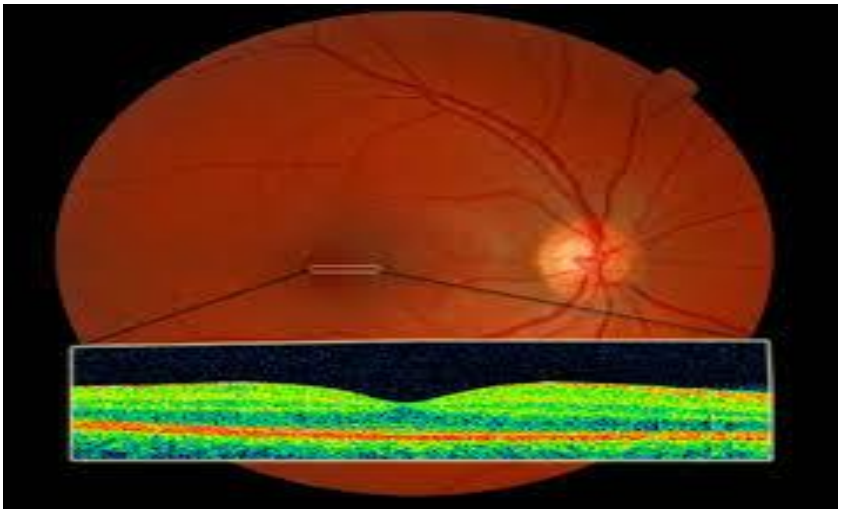
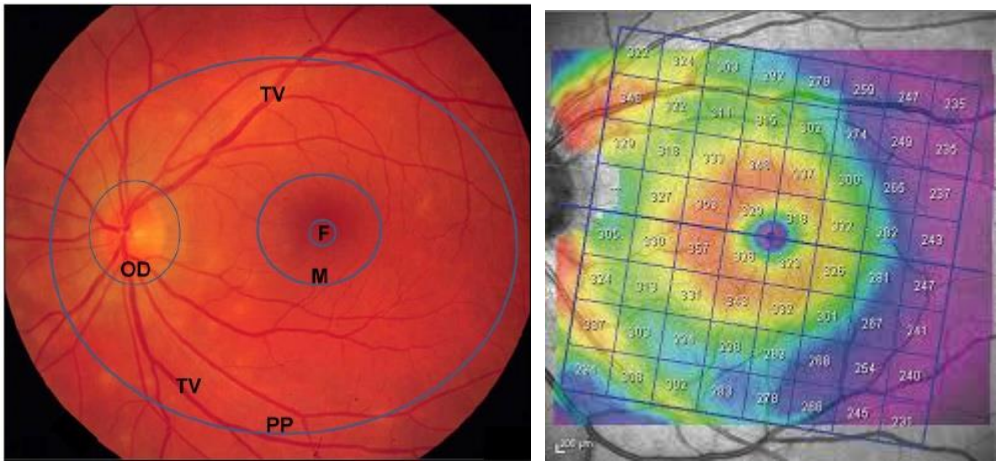


Fig: 7.2 showing posterior pole and correlating OCT image.



Chapter 8

Structure meets function

Result 1: Evaluation of Visual function test

Chapter 8: Results

8.1. Study 1: Evaluation of Visual function test in disease and control

Introduction

We methodically conducted functional assessment of visual pathway using the standard Snellen's vision charts comparing with the Logarithmic minimal angle of resolution chart (LogMAR) and sensitive Sloan low contrast (2.5% & 1.25%) visual acuity charts.

8.2. Methodology

A total of 60 patients (mean age 48, range 32-68) and 25 controls (mean age 45, range 38-52) were considered for the study. 5 patients from the disease arm were considered un-suitable (See figure 8.1). Thus, one hundred and ten eyes with MS and 50 eyes, disease-free control subjects participated in a single testing session. There were few exceptions to the single testing process, which accounted for six patients, who were recalled due to poor focus and inability to concentrate as a part of the disease process.

Figure 8.1: Flow chart of patient recruitment

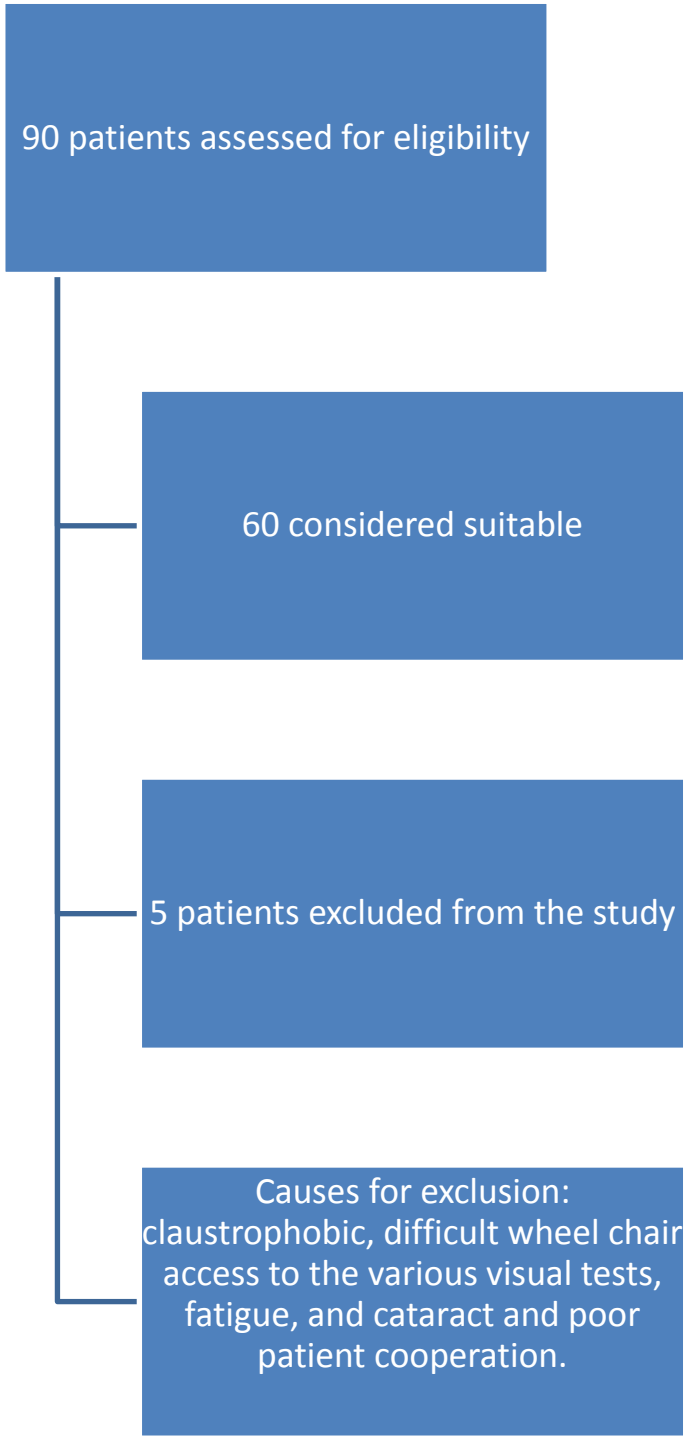


Table 8.2: Baseline features of recruited candidates.

	Total patients N	Mean age in years (Range)	Male: Female ratio	Ethnicity	RRMS	SPMS
Control	25	45 (38-52)	40:60	Caucasian 100%	0	0
Disease	55	48 (32-68)	30:70	100% Caucasian	55	0

RRMS: Relapsing-Remitting MS; SPMS: Secondary Progressive MS;

N= patients recruited.

Table 8.3: Neurological sub-grouping of disease

Median EDSS Disability score	Median disease duration (years)	Patients on DMT	Patients not on DMT	ARR (relapse rate in prior 24 months
3.2+_ 2.8	8.2 +_ 8.4	25 (50 eyes)	30 (60 eyes)	0.4+- 0.8

EDSS: Expanded Disability Status Scale; DMT: Disease modifying treatment

ARR: Annualised Relapse Rate.

Table 8.4: Optic neuritis subgroup

Total eyes In disease group N	MS with No history of optic neuritis	MS-with history of optic neuritis	Bilateral ON	Unilateral ON	Median Age at ON (years)	Duration of ON (years)
110 eyes	65 eyes	45eyes	26 eyes (13 patients)	19 eyes	22 years (18-35)	18 years (10-28)

N: number; MS: multiple sclerosis; ON: optic neuritis

Patients and controls underwent testing with the standard chart (Snellen's) and LogMAR (100%) and Sloan 2.5%, 1.25% charts. All patients were tested for refraction and need for glasses. A focimeter helped to determine refraction in patients with existing glasses.

Snellen's chart:

Most patients in the disease and control arm performed the test with ease. Results from the test charts were obtainable in all patients with no hindrance from patient or test factors. Overall letter score as depicted by the Table 8.5 below showed no significant difference in the disease versus control arm. The optic neuritis group missed few more letters when compared to the overall disease group.

Table 8.5: Snellen's letter score in the different groups

	Snellen's binocular letter score Mean (standard deviation)
Control (n= 25 patients)	56 (SD 2)
Disease (n= 55 patients)	53 (SD 3
Optic neuritis group(n=45 patients)	49 (SD 2)
DMT group(n=25 patients)	53 (SD 3)
No optic neuritis group 65 patients	53 (SD 2)

Distribution analysis of visual letter score charts for LogMAR and Sloan.

Depicted is the distribution of letter scores derived in the disease cohort.

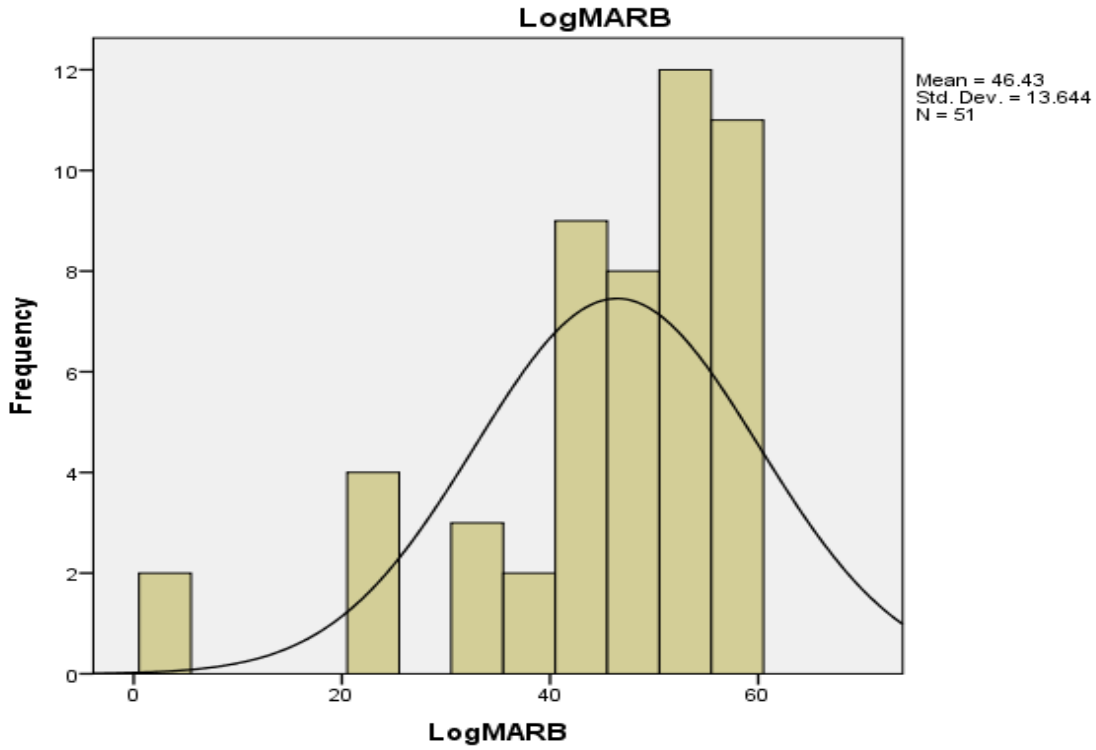


Figure 8.7: distribution of data of the disease group on binocular LogMAR charts

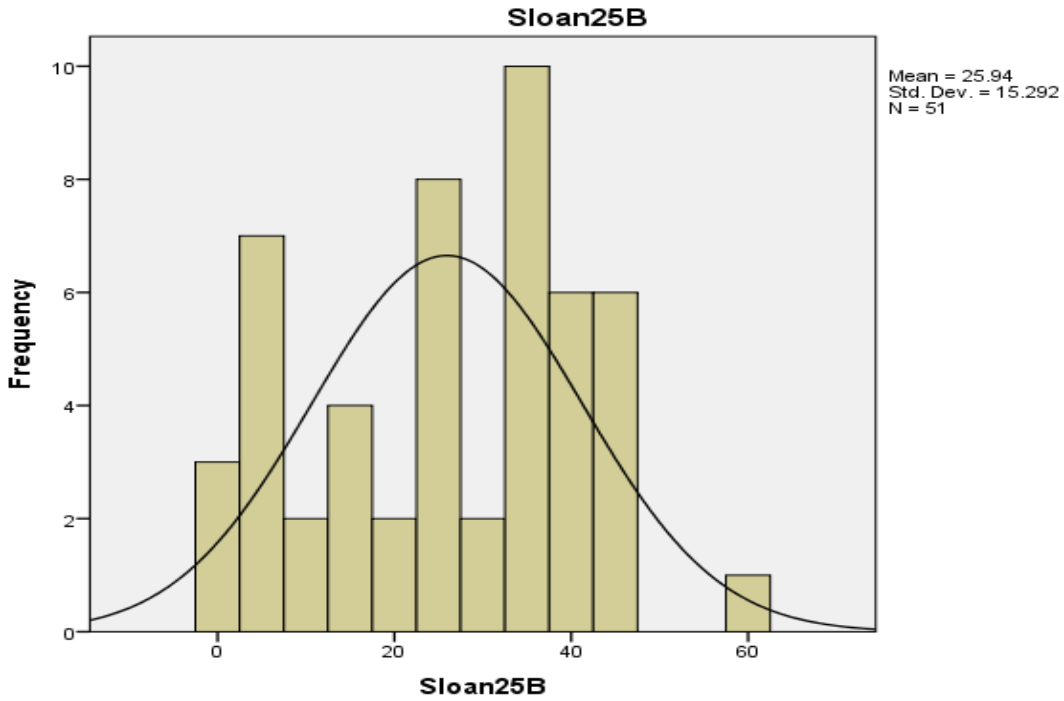


Figure 8.8: distribution of data of the disease group on binocular Sloan 2.5%charts

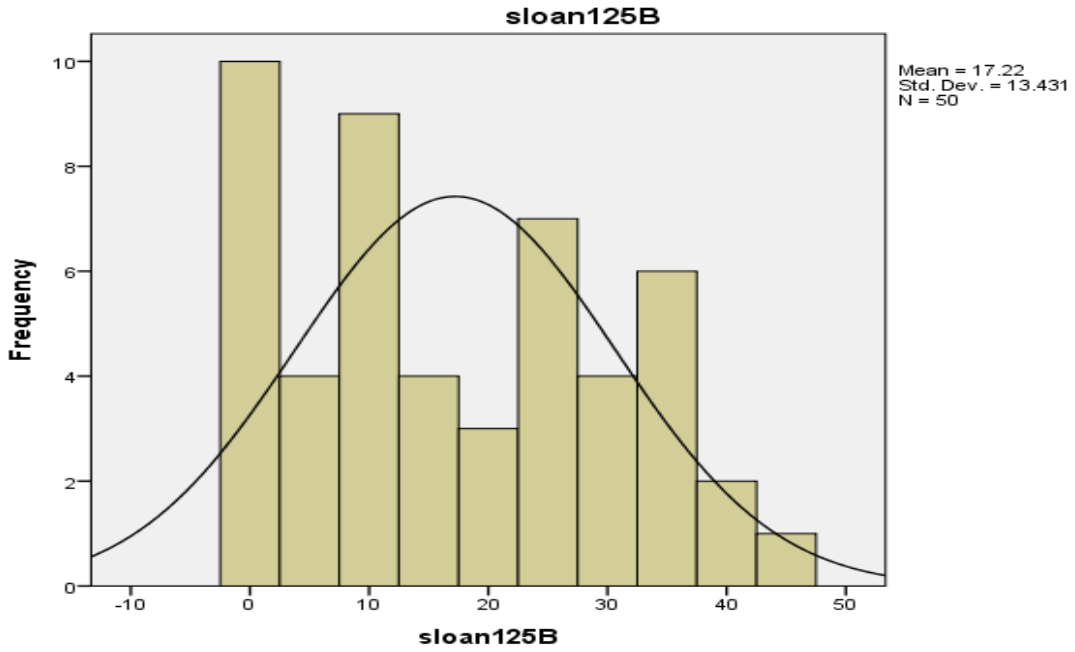


Figure 8.9: distribution of data of the disease group on binocular Sloan 1.25% charts

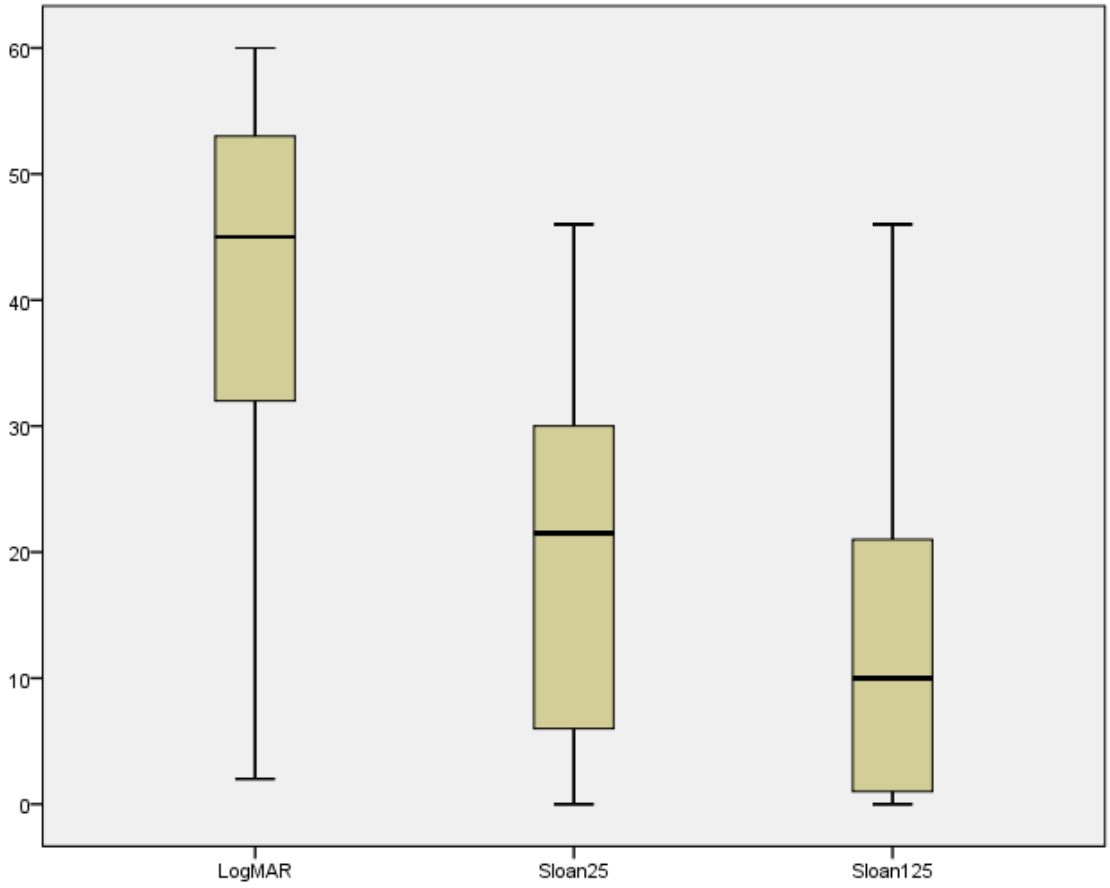
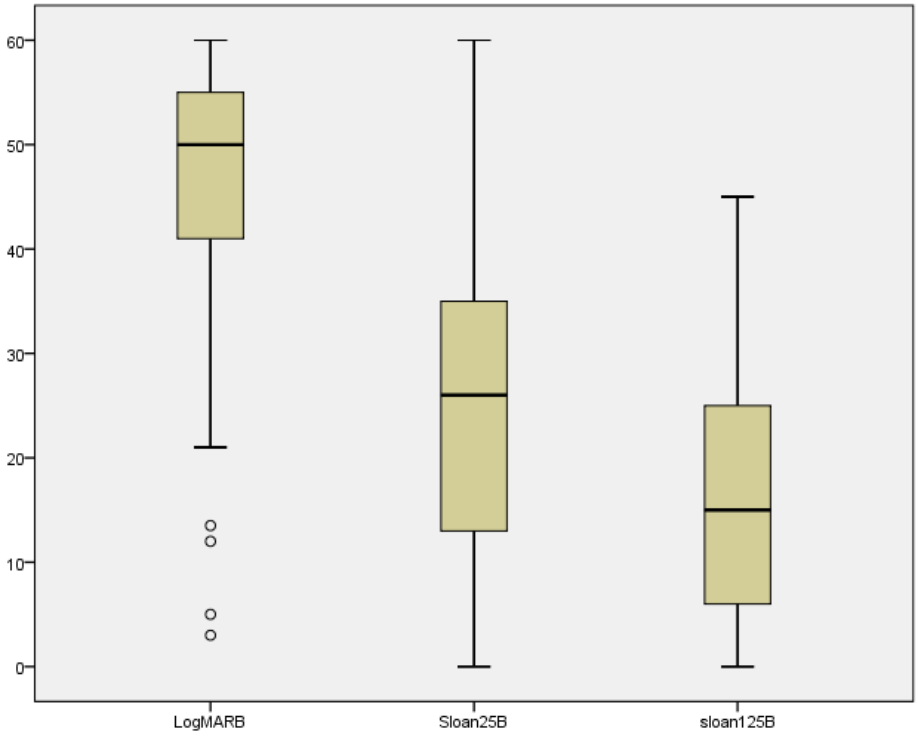


Figure 8.10: Box- whisker plot showing IQR for LogMAR, Sloan 2.5% and Sloan 1.25% charts tested uniocularly for the right eye in the disease arm. Y axis shows letter scores for each variable.

Figure 8.11: Box- whisker plot showing IQR for LogMAR, Sloan 2.5% and Sloan 1.25% charts tested binocularly in the diseased arm. Y axis shows binocular letter scores for each test chart

X axis shows different test chart



Vertical bars indicate inter-quartile range (25th to 75th percentile observations) for each median score. Lower contrast levels (%) correspond to lighter gray letters on the white background. Despite nearly equal scores for visual acuity (difference in median scores of 2 letters), greater differences were observed for contrast letter acuity scores at lower contrast levels, particularly 1.25% ($p = 0.0001$ for all levels). These differences were noted even following correction of refractive error in both the MS and the control groups

Sloan 2.5% and 1.25% charts

The Sloan chart is used similarly to a LogMAR chart with the same standard illumination and standard distance of 4 meters. The only difference is the contrast of letters, which decreases in the row and between the rows of letters. There was a highly significant difference between the 2 groups in the 2.5% and 1.25% letter score. See chart below (Figure 8.6)

There was a clear struggle to read the letters on the 1.25% and 2.5% chart in the disease group when compared to the control arm. The struggle to read was worse in the 1.25% group. This has sometimes been attributed to floor lighting in some previous articles, though clearly this was not the case in our cohort.

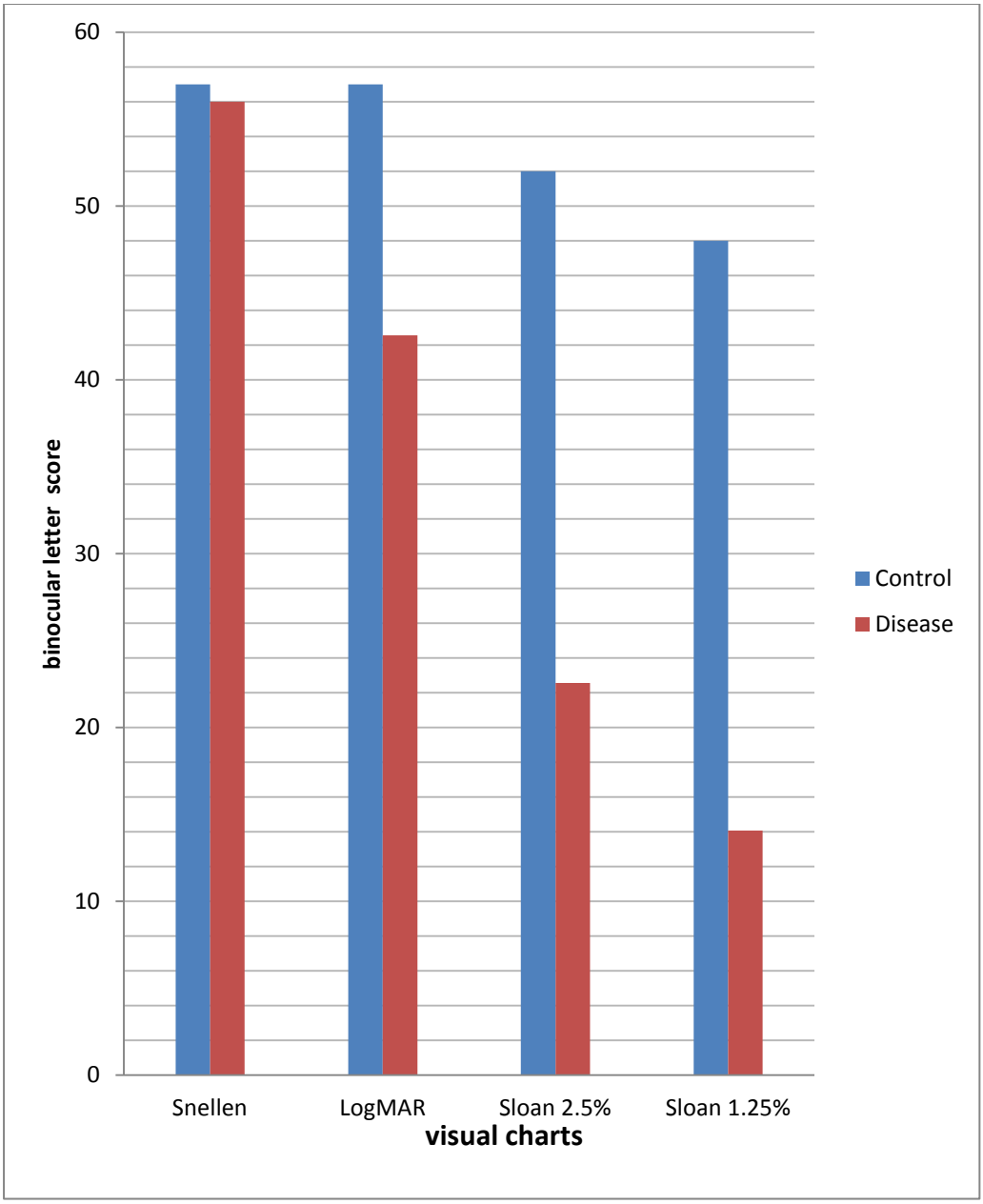


Figure 8.6: Bar chart representing the sensitivity of different chart in the same subset of patient and control. (Sloan charts showing increased detection of patients affected with optic neuropathy in disease state)

Binocular testing

8.6. Concept of binocular summation

During the vision testing, both uniocular and binocular vision was tested. In our cohort, there was increase in the binocular letter scores. Binocular summation of acuity occurs when vision is improved under binocular viewing conditions (binocular score is greater than the scores for either eye alone) (Figure 8.12)

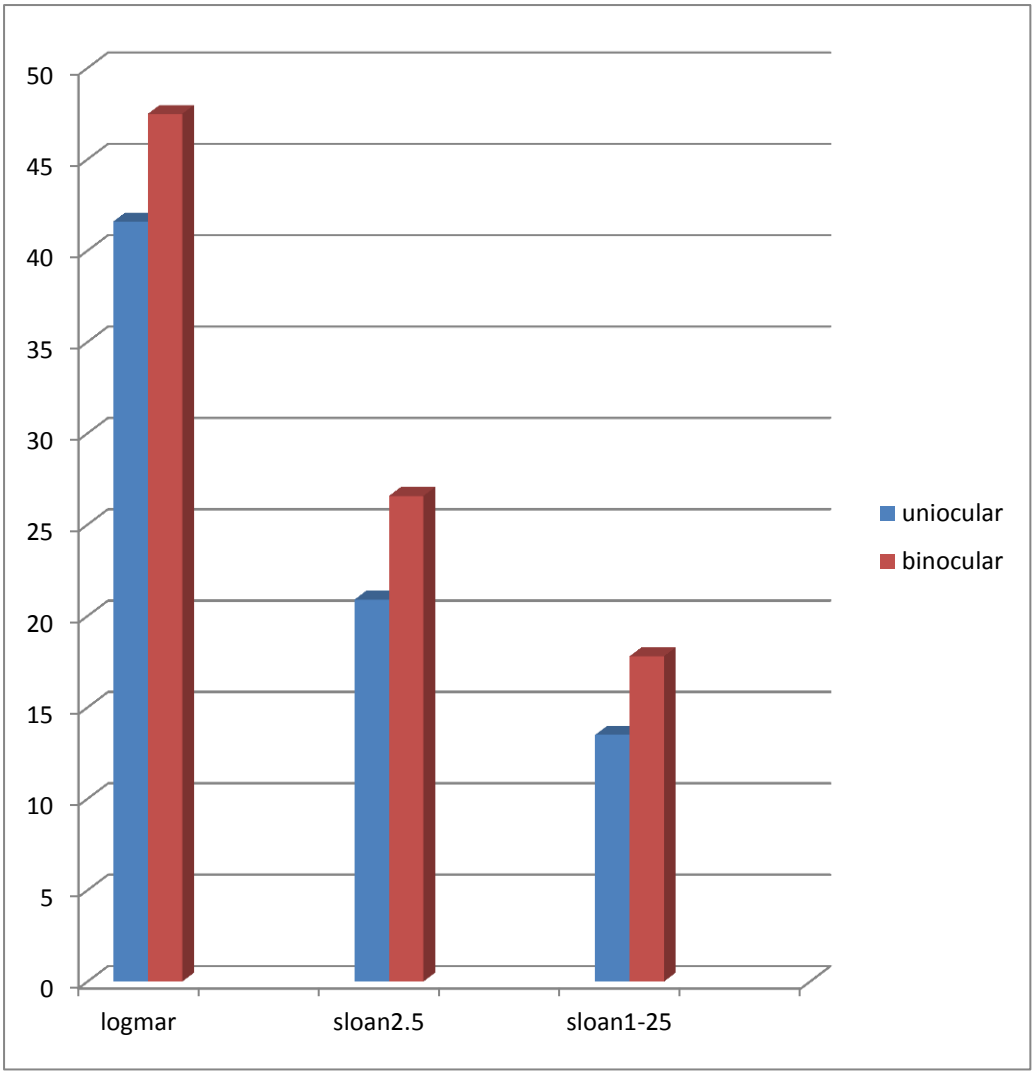


Figure 8.12: Showing binocular summation to different vision chart

8.7. Concept of Binocular inhibition:

Patients with binocular inhibition had worse binocular vision compared to the better eye alone. Among 55 patients with MS and 25 disease-free controls, binocular summation was substantial (5–7-letter increases over better eye acuity, 28–52% with>7 letters, P<0.001) for low-contrast acuity at the 2.5% and 1.25% levels. For high-contrast VA, only 3.0–3.4% of patients showed similar degrees of summation. Increasing age (P<0.0001), greater interocular differences in acuity (P<0.0001), and prior history of ON (P=0.015) were associated with lower magnitudes of binocular summation; in fact, 2 of these patients had binocular inhibition. This mainly was linked to the severity of optic neuritis along with age at optic neuritis. Both patients had optic neuritis after 30 years of age and visual deficit was severe at the time. This area need addressing for future studies as it can improve quality of life and vision in Multiple sclerosis.

8.8. Vision in optic neuritis group

Vision was further studied in the positive history of optic neuritis MS group and the no history of optic neuritis MS group (NON MS). Data was filtered for the 2 different groups using the split data function and thereafter group comparatives descriptive were used. The data was derived from the most consistent group and outliers were not considered. The results are presented below.

Table 8.11: Descriptive Statistics OPTIC NEURITIS= NO

	N	Minimum	Maximum	Mean	Std. Deviation
LogmarU	65	12	60	43.31	13.135
Sloan25U	65	0	46	24.25	13.178
Sloan125U	65	0	46	15.16	12.326
Valid N	65				

Table 8.12:Descriptive Statistics OPTIC NEURITIS= YES control stats

	N	Minimum	Maximum	Mean	Std. Deviation
LogmarU	42	2	55	36.27	17.548
Sloan25U	42	0	45	14.08	13.056
Sloan125U	42	0	45	7.22	10.912
Valid N	42				

Table 8.13: Descriptive statistics for Severe Optic neuritis

Total eyes 3	Minimum Letter score	Maximum Letter score	Mean Letter score	Std. Deviation
LogmarU	30	45	35.00	8.660
Sloan25U	0	33	14.67	16.803
Sloan125U	0	22	7.33	12.702

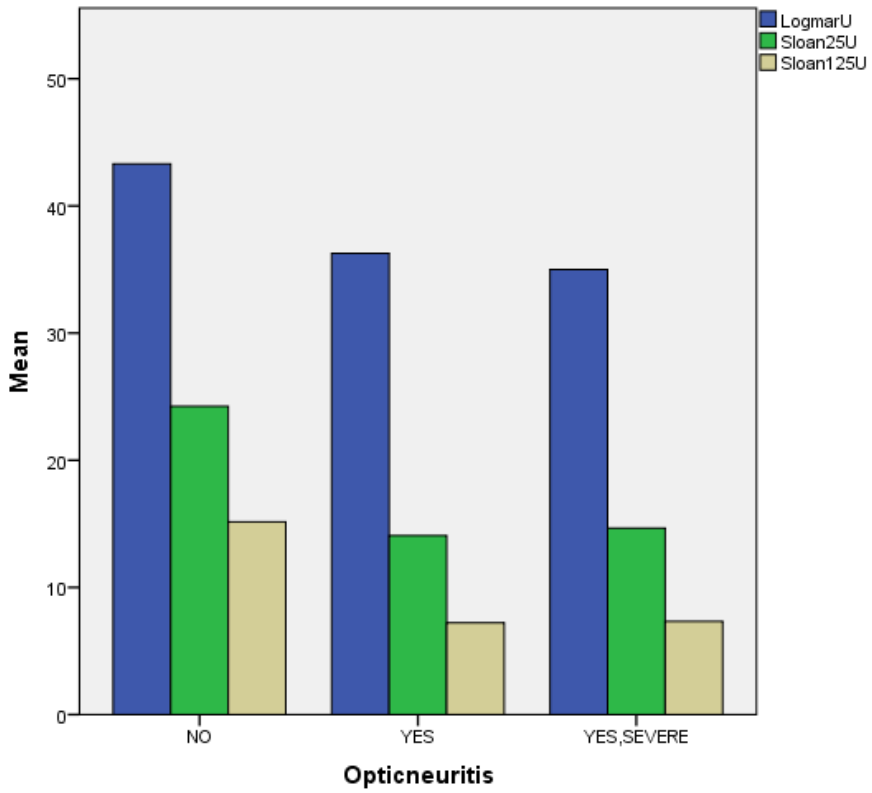


Figure 8.14: Bar chart depicting letter score on LogMAR, Sloan 2.5% and 1.25% chart in patients with no, yes and severe optic neuritis group

8.9. Sloan letter acuity chart in patients on DMT (disease modifying treatment)

Table 8.15:Descriptive Statistics of patients on **NO** treatment

Binocular	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
SLOAN25	30	0	45	28.05	13.923	-.622	.512
SLOAN125	30	0	45	21.10	13.034	.109	.512
Valid N	30						

Table 8.16: Descriptive Statistics of patients **with** treatment

Binocular	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
SLOAN25	25	0	60	24.58	16.190	.007	.421
SLOAN125	25	0	35	14.63	13.273	.456	.427
Valid N	25						

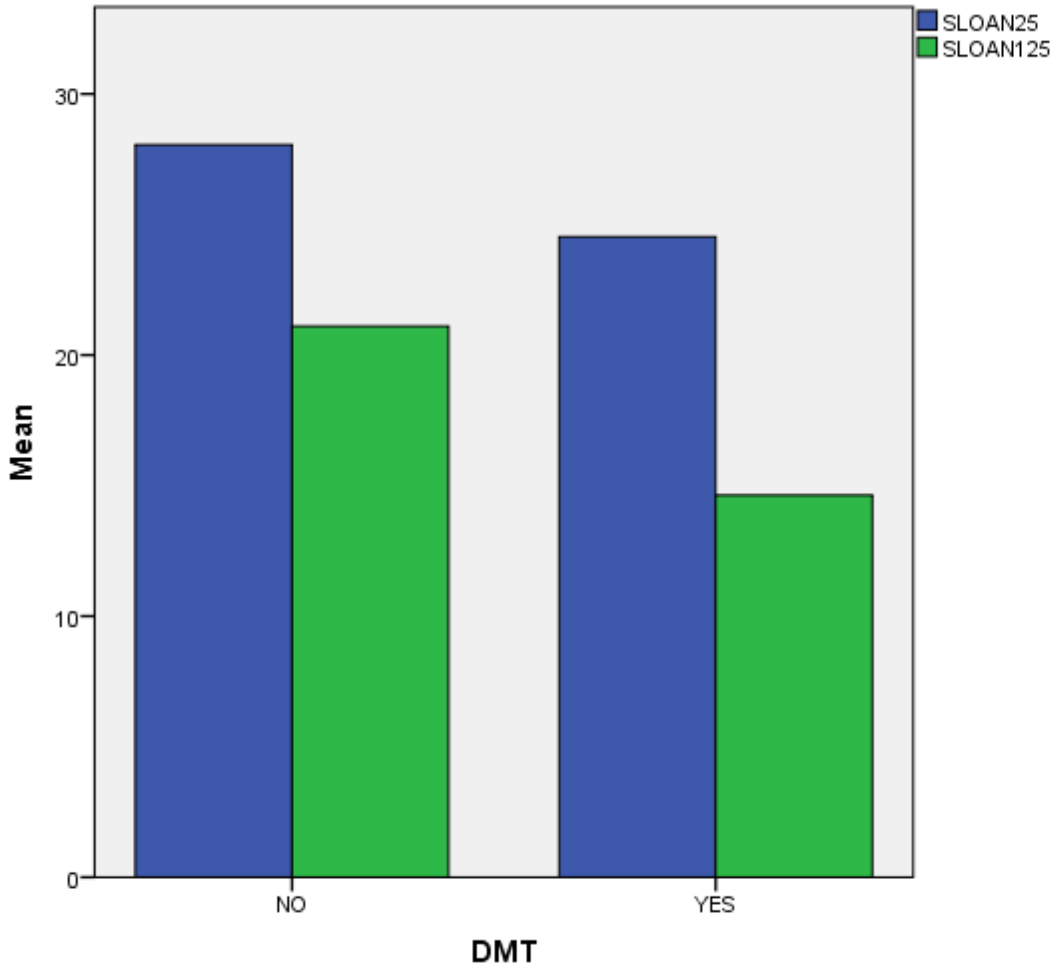


Figure 8.17: Clustered bar chart depicting letter scores on Sloan 2.5% and Sloan 1.25% contrast chart in the treated versus untreated group.

8.10. Comparison of visual function tests using ROC curve analysis

Visual function test (units) ROC curve area (95% CI)

The ROC curve area represents the probability that each test will correctly identify the cohort participants as MS patients versus disease free controls. The areas range from 0.5 (no ability to distinguish Multiple sclerosis) to 1.0 (perfect capacity to distinguish).

The vision measure with the greatest area under the ROC curve was considered the test that best distinguishes MS participants from controls on the basis of binocular visual function. Using binocular scores, low-contrast letter acuity (1.25% charts) had the greatest capacity to distinguish all MS patients from controls using logistic regression and ROC areas (ROC area 0.412, p=0.006). In comparison, ROC areas were decreased and did not distinguish between healthy controls and MS participants using binocular high-contrast acuity, 2.5% low-contrast

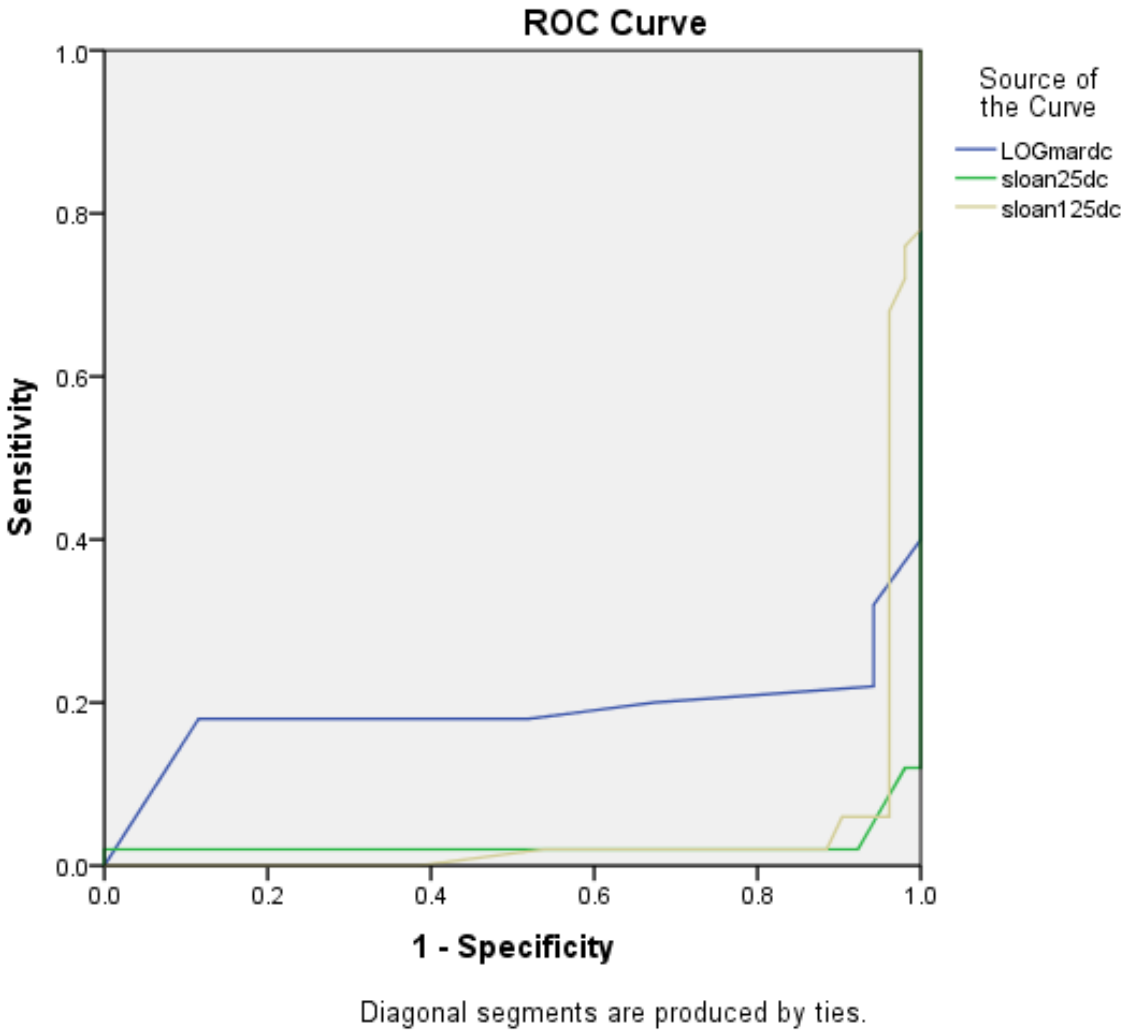


Figure 8.18: ROC curve for different test charts check curve + CI

The test result variable(s) has at least one tie between the positive actual state group and the negative actual state group. Sloan 1.25% chart is more specific in detecting the disease state compared to the other test charts.

Table 8.19: Area Under the Curve

Test Result Variable(s)	Area
LogMAR	.190
Sloan 2.5	.025
Sloan1.25	.041

Chapter 9

Result 2: Evaluation of colour vision in disease and control

Colour vision in MS

Multiple Sclerosis (MS) frequently causes injury to the anterior visual pathway (AVP), impairing quality of life due to visual dysfunction. Although the mechanisms involved in colour vision impairment are not completely understood, the results of functional tests of colour vision in our study suggests that impaired colour vision is produced primarily by injury to the anterior visual pathway.

Colour vision involves the detection of light spectra by colour-sensitive cones (red, green and blue light) that project to bipolar to retinal ganglion cells (RGCs). Axons from RGCs then project to Parvocellular and Koniocellular neurons in the lateral geniculate nucleus (LGN), which in turn send projections to the V1 region of the visual cortex.

Hardy, Rand and Rittler (HRR) pseudoisochromatic plates allow the presence of acquired colour vision impairment to be evaluated at the patient's bedside within a few minutes

We sought to investigate whether assessments of colour vision augment measures of visual disability in MS. We studied the association between high-contrast visual acuity charts, LCVA and colour vision using HRR (Hardy-Rand-Rittler plates). This will later be correlated with findings of OCT, using high-resolution spectral-domain OCT (SD-OCT, new generation).

The group of 55 MS patients and 25 controls underwent Ishihara and HRR colour testing methods. We also sub-classified the group with previous optic neuritis and the group receiving disease-modifying treatment in this cross sectional study.

Controls:

Twenty-five normal controls were recruited from the clinic staff and from relatives accompanying patients. After obtaining informed consent, 10 males and 15 females, aged 32to 53, were questioned for a history of colour blindness in themselves or in a primary relative, for a history of visual disturbance, and for the presence of a systemic illness that could affect vision. Following confirmation that there were no known visual disturbances, control subjects underwent the same series of tests as did the MS patients.

9.2. Colour vision testing using Ishihara test charts

Ishihara's Tests for colour blindness (ITCB) is a collection of pseudo-isochromatic plates that measures colour vision and is largely independent of pre retinal changes .The complete edition of ITCB contains 38 plates, while a concise edition, assembled in 1989, consists of a subset of 14 plates. The first 11 plates of the concise edition, identifying patients with red-green colour deficiency, were used in this study. Misreading up to 1 or 2 plate out of 11 was considered normal.

Table 9.1: Ishihara testing outcome

Plate	Control RE	Control LE	Control BE	MS RE	MS LE	MS BE
1	0	0	0	0	0	0
2	0	0	0	1	0	1
3	0	0	0	0	0	0
4	0	0	0	2	1	1
5	1	0	1	3	2	4
6	0	0	0	1	0	0
7	0	0	0	0	1	0
8	1	1	1	2	6	2
9	0	0	0	0	0	0
10	0	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0
Total	2/25	1/25	2/25	9/55	10/55	8/55

9.3. HRR colour chart

Colour vision was then tested using Hardy, Rand and Rittler (HRR) pseudoisochromatic plates. The HRR test has 24 plates with 0, 1 or 2 symbols depending of the plate. There are three types of plates: 4 non-scored demonstration plates; 6 scored screening plates and 14 scored plates for type and severity assessment. The first four plates (plates 1–4) are used to ensure understanding and enough visual acuity to perform the task. The 6 screening plates (plates 5–6 with 4 symbols for blue-yellow deficit and plates 7–10 with 6 symbols for red-green) classify eyes as having dyschromatopsia or normal colour vision. An eye with less than two errors is considered normal. There is no need to continue the test if the HRR score is 35 (one error) or 36 (zero errors). We used two errors as a cut-off point to ensure a sensitivity of 1.0, as has been previously described. An eye with two or more errors in the screening plates has impaired colour vision and plates 11–24 were administered to determine type and extent of the defect. Plates 11–20 (18 symbols) and plates 7–10 are used to assess red-green defect with three levels of severities: mild (plates 7–10 with 6 symbols and plates 11–15 with 8 symbols); moderate (plates 16–18; 6 symbols) and severe (plates 19–20, 4 symbols). Plates 21–24 (8 symbols) and plates 5–6 are used to evaluate blue/yellow defect

with three levels of severities: mild (plates 5–6); moderate (plates 21–22; 4 symbols) and severe (plates 23–24; 4 symbols). The last group of plates in which errors occur establishes the extent of the color deficit. For instance, if the last error occurs in either plates 7–10 or 11–15 and there are no errors in plates 16–20 there is a mild red-green deficit. In our study, color vision is evaluated qualitatively based on the number of errors in the screening plates: an eye has a color vision deficit if it has at least two errors in the screening plates. Moreover, color vision was measured quantitatively based on the number of correctly identified symbols in the 20 scored HRR plates with a maximum of 36 symbols.

Statistics

This study collects a wide range of results that was examined using the following standard approach. First, we performed descriptive statistics to characterize the sample using absolute numbers and proportions for qualitative variables and mean and standard deviation for quantitative variables. Data was tested for normality, homoscedasticity and independence to test assumptions used for parametric tests. Then, we performed bivariate analyses using X^2 test or Fisher test (in case of small samples) for qualitative variables and independent 2-sample t-test or ANOVA (three or more groups) for quantitative variables. For data that is not parametric, we will use the U-Mann–Whitney test. Pearson's Correlation test or the non-parametric analogue, Spearman rank order correlation coefficient, will be used to evaluate correlations. Finally, we will

perform multivariate tests to rule out confusion that is inherent to cohort studies. Two-tailed p-values <0.05 will be considered statistically significant. All analyses were performed with the Statistical Package IBM-SPSS software version 21V

9.4. Comparative data ITCB and HRR

Table 9.2: Defective plates read in Ishihara versus HRR chart (mean)

Defective	IHRC	IHRD	HRRC	HRRD
Right	2	9	2	16
Left	1	10	3	15
Both	2	8	5	14

IHRC : Ishihara for controls, IHRD: Ishihara for disease, HRRC: HRR chart for control, HRRD: HRR for disease

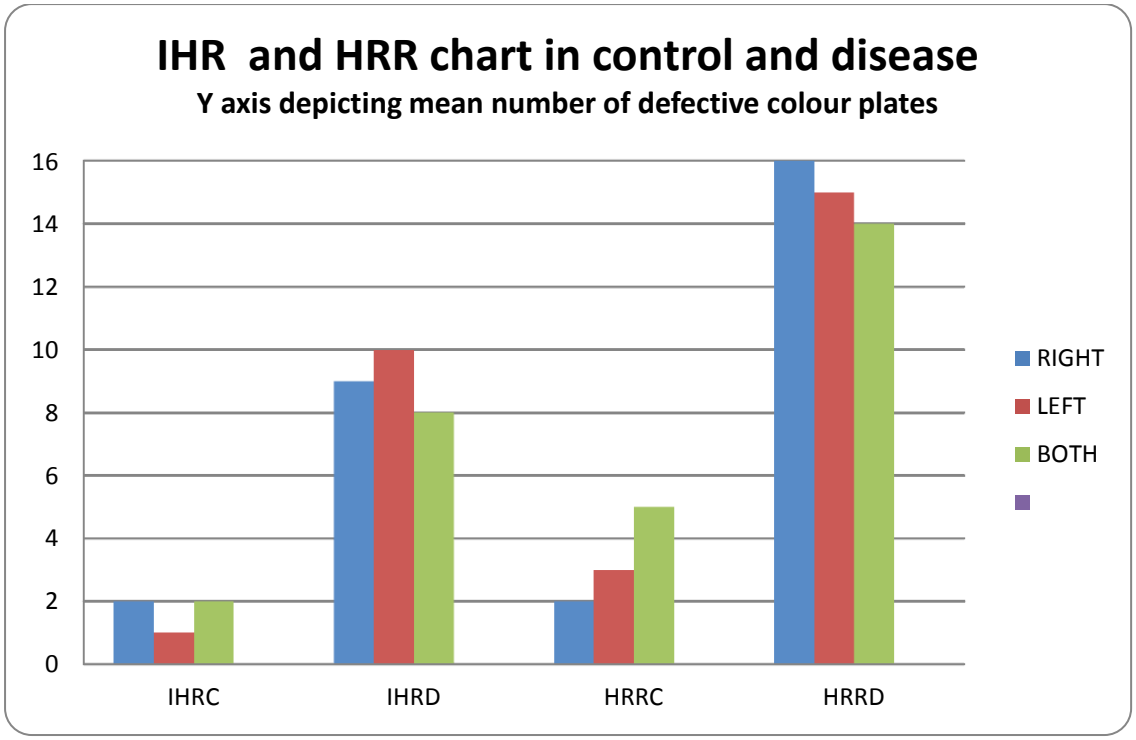


Figure 9.3: Bar chart depicting the detection of colour deficit using IHR and HRR charts in control and disease group. Y-axis shows the number of colour plates unable to read in right , left and both eyes.

The HRR chart proved to be more sensitive to damage of the retinal ganglion cells, reflected by the extent of detection of colour vision deficit compared to the Ishihara charts. In addition, the HRR chart has the potential to classify these groups into mild, moderate and severe colour deficit along red –green and blue-yellow axis. This may form an indirect clue to affection of the Magnocellular and Parvocellular pathway.

Table 9.4: HRR testing outcome:

HRR control

Defective colour vision	IHR RE	IHR LE	IHR BE	HRR RE	HRR LE	HRR BE
Controls (n= 25)	6	4	5	0	0	0
Disease (n=55)	14	10	16	16	15	14
Mild RG	-	-	-	10	9	10
Mod RG	-	-	-	4	4	2
Severe RG/ BY	-	-	-	2	2	2
Normal	11	15	9	39	40	41?

Table 9.5: Detection of severity of colour blindness

	HRR RE	HRR LE	HRR BE
Mild RG	10	9	10
Mod RG	4	4	2
Severe RG/ BY	2	2	2

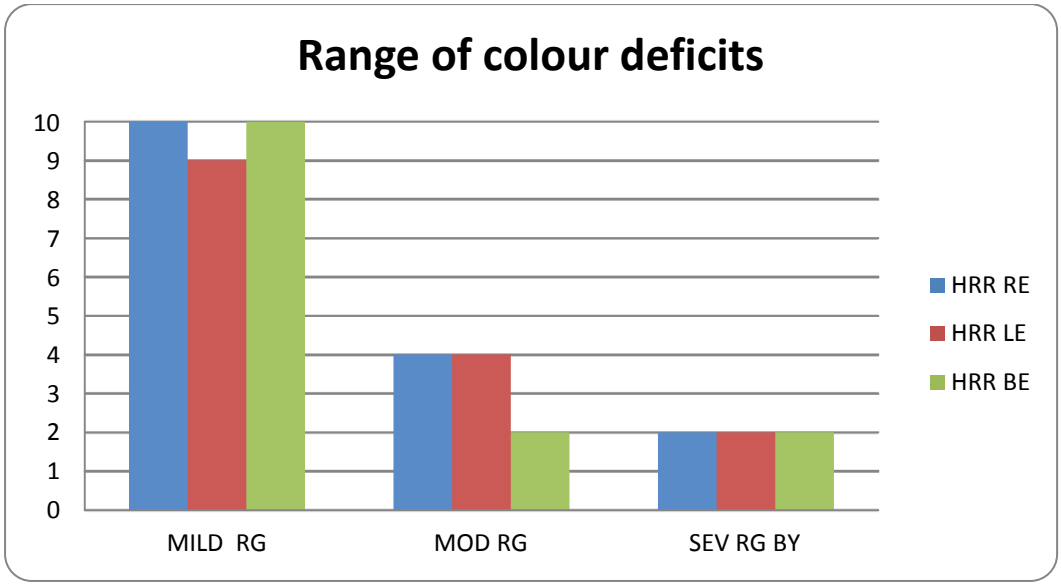


Figure 9.6: Colour deficit in the cohort of study patient (disease arm)

The table below shows the spread of data in the MS arm of the study. 60% of patients had no history of optic neuritis while 40% had history of optic neuritis with severe symptoms in 2.7% patients recruited.

Table 9.7: showing the number of cases with no history of optic neuritis(N), history of optic neuritis (Y) and severe optic neuritis(Y SEV)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	N	65	60.0	60.0	60.0
	Y	42	37.3	37.3	97.3
	Ysevere	3	2.7	2.7	100.0
	Total	110	100.0	100.0	

This can be graphically represented by a bar chart for better understanding.

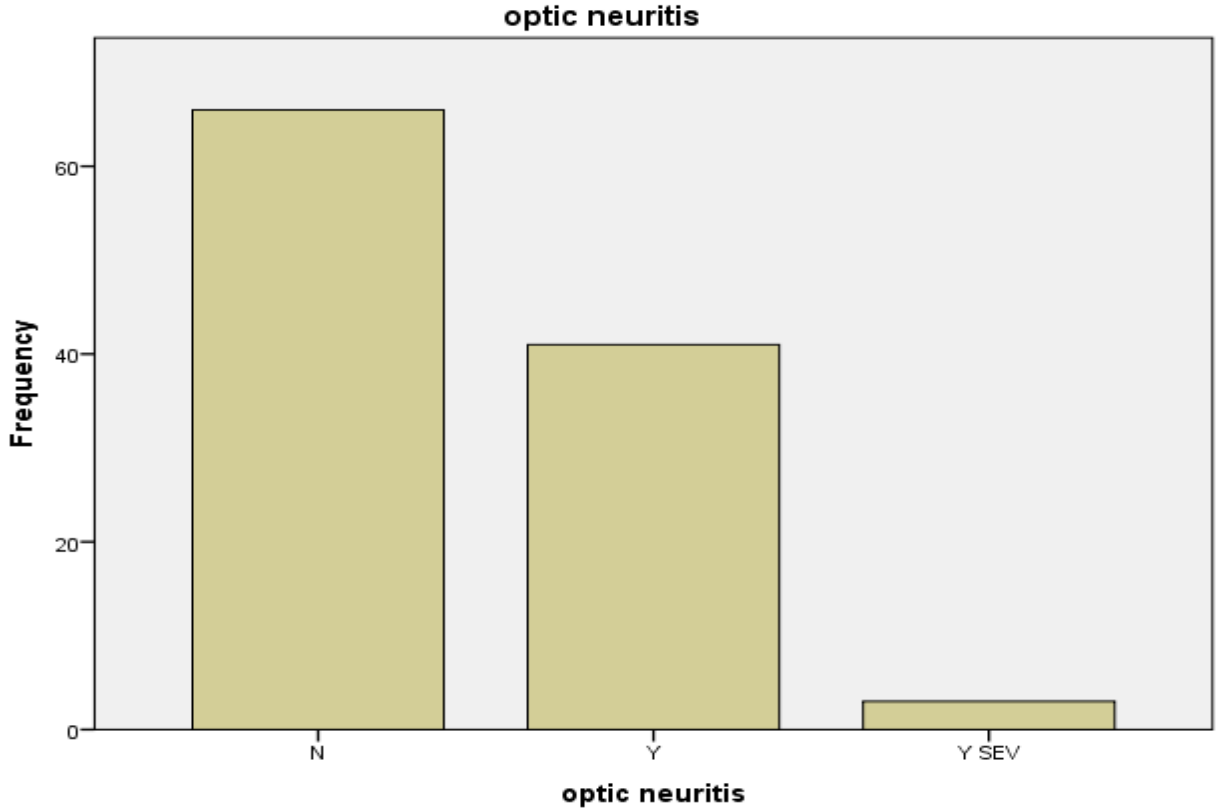


Figure 9.7a: Bar chart depicting the visual outcome in different optic neuritis groups.

Study group and colour blindness

Table 9.8 shows patients with normal colour vision to range of colour deficits.

Table 9.8:Extent of colour blindness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Mild BY	1	.9	.9	.9
	Mild RG	19	17.3	17.3	18.2
	Mod BY	2	1.8	1.8	20.0
	Mod RG	8	7.3	7.3	27.3
	Normal	76	69.1	69.1	96.4
	Severe RG/BY	4	3.6	3.6	100.0
	Total	110	100.0	100.0	

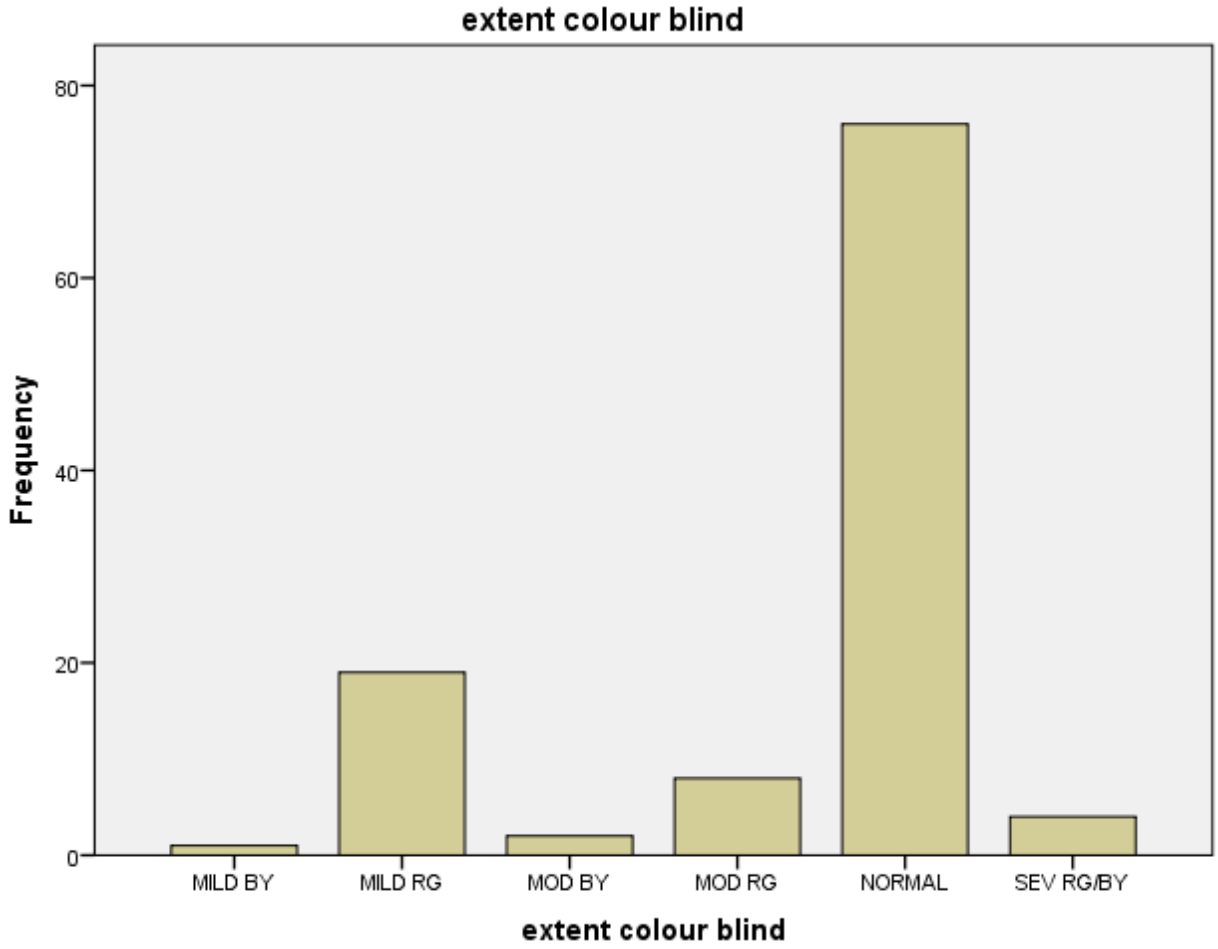


Figure 9.8a: Bar chart showing the range of colour deficits in the disease group.

The data was then grouped into the optic neuritis group with positive history (yes group) and the other with no history of ON (no group). The data split function on SPSS Vs 21 was used and the frequencies at which patients suffered with colour deficit in the 2 groups were studied.

Interesting results were revealed as shown in the charts.

A) The negative ON history group:

66 patients had no history of optic neuritis. Hence we probably would expect the colour vision to be normal in this group. On the contrary, this group showed a variety of colour deficit which ranged from mild red green to severe red green deficiency and from mild blue yellow deficit to severe blue yellow deficit in colour vision.

This opens a new concept of asymptomatic or sub-clinical loss of colour vision in Multiple sclerosis.

Statistics

a. No Optic neuritis N=65

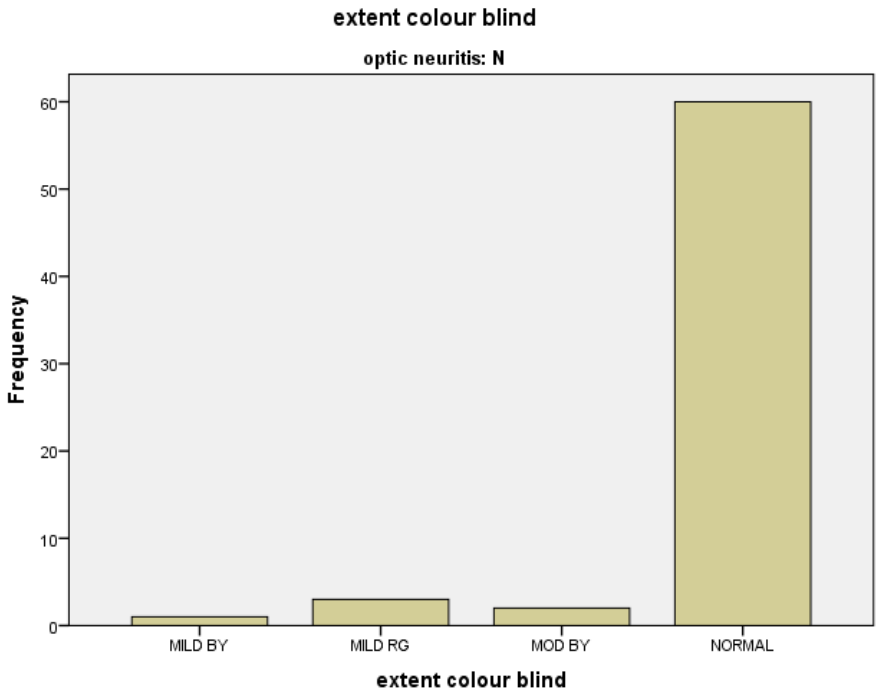


Figure 9.9: Colour deficits in the disease group with no history of optic neuritis

B) The positive history of optic neuritis group

The positive history of optic neuritis was then studied. There were 42 eyes in this group with 28 eyes suffering from mild to severe colour abnormalities termed as dyschromatopsia. We would expect a majority of the group to have some form of dyschromatopsia, though this was not the case. We had 14 eyes (32%) with no detectable colour abnormalities.

Statistics

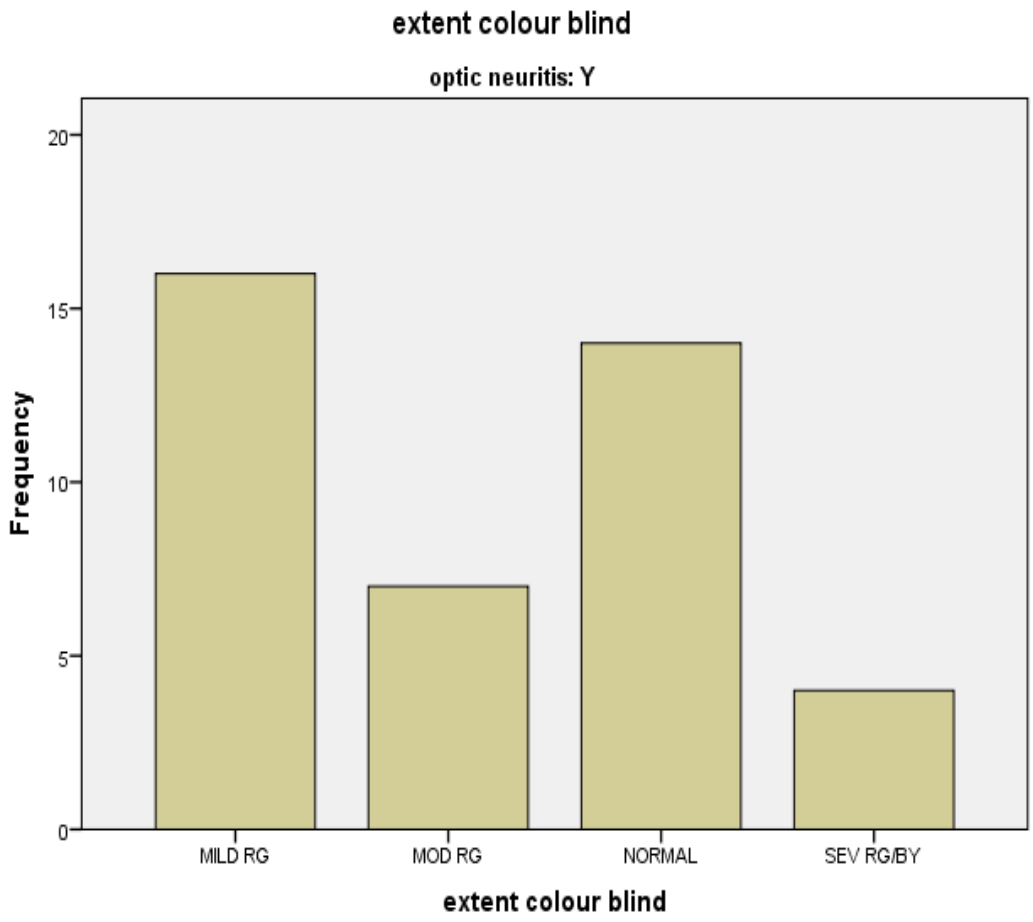
a. Optic neuritis = Yes group

N	Valid	42
	Missing	0

		Total eyes	Percent eyes	Valid Percent	Cumulative Percent
Valid	Mild RG	16	39.0	39.0	39.0
	Mod RG	8	17.1	17.1	56.1
	Normal	14	34.1	34.1	90.2
	Severe RG/BY	4	9.8	9.8	100.0
	Total	42	100.0	100.0	

a. Optic neuritis = Yes group

Figure 9.10: Bar chart showing the frequency of normal colour vision to dyschromatopsia.



C) The group with severe optic neuritis

Statistics

extent colour blind

N	Valid	3
	Missing	0

a. Optic neuritis = Yes, Severe group

Figure 9.11: Bar chart showing severe colour deficit in positive history of optic neuritis group.

extent colour blind

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Moderate RG	1	33.3	33.3	33.3
	Normal	2	66.7	66.7	100.0
	Total	3	100.0	100.0	

a. Optic neuritis = Y SEV

Further the categorical data in the 2 groups was compared using a chi-squared test. The results are shown in Table 9.12.

Interpretation:

The results of a chi-square test, X2 (chi-square) value is 0.543 with 10 degrees of freedom for a sample size of 110. The exact significance level was 0.000, which meant that the 95% confidence level was merely a chance with a highly significant chi square value.

Table 9.12: Range of colour deficits in the 3 subgroups

optic neuritis * extent colour blind Crosstabulation

		extent colour blind						Total
		MILD BY	MILD RG	MOD BY	MOD RG	NORMAL	SEV RG/BY	
optic neuritis	No	1	3	2	0	60	0	66
		100.0%	15.8%	100.0%	0.0%	78.9%	0.0%	60.0%
	Yes	0	16	0	7	14	4	41
		0.0%	84.2%	0.0%	87.5%	18.4%	100.0%	37.3%
	Yes severe	0	0	0	1	2	0	3
		0.0%	0.0%	0.0%	12.5%	2.6%	0.0%	2.7%
Total		1	19	2	8	76	4	110
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 9.13: Chi square on the 3 groups

	Value	df	Sig. (2-sided)
Pearson Chi-Square	51.483[a]	10	.000
Likelihood Ratio	57.080	10	.000
McNemar-Bowker Test	.	.	.[b]
N of Valid Cases	110		

a. 14 cells (77.8%) have expected count less than 5. The minimum expected count is .03.
b. Computed only for a PxP table.

Table 9.14 : correlation coefficient

		Value	Approx. Sig.
Nominal by Nominal	Phi	.684	.000
	Cramer's V	.484	.000
N of Valid Cases		110	

9.6. Logmar , Sloan , colour chart and its correlation explored

Visual function charts (LogMAR) testing form sense was compared with charts to test contrast (Sloan 2.5 and Sloan 1.25%) and colour (HRR)chart . As a rule of thumb, scatter plots for each of the data set was used to explore the best-fit line and subsequent analysis for correlation.

Figure 9.15:Scatter plot of LogMAR and HRR colour chart data(both eyes)

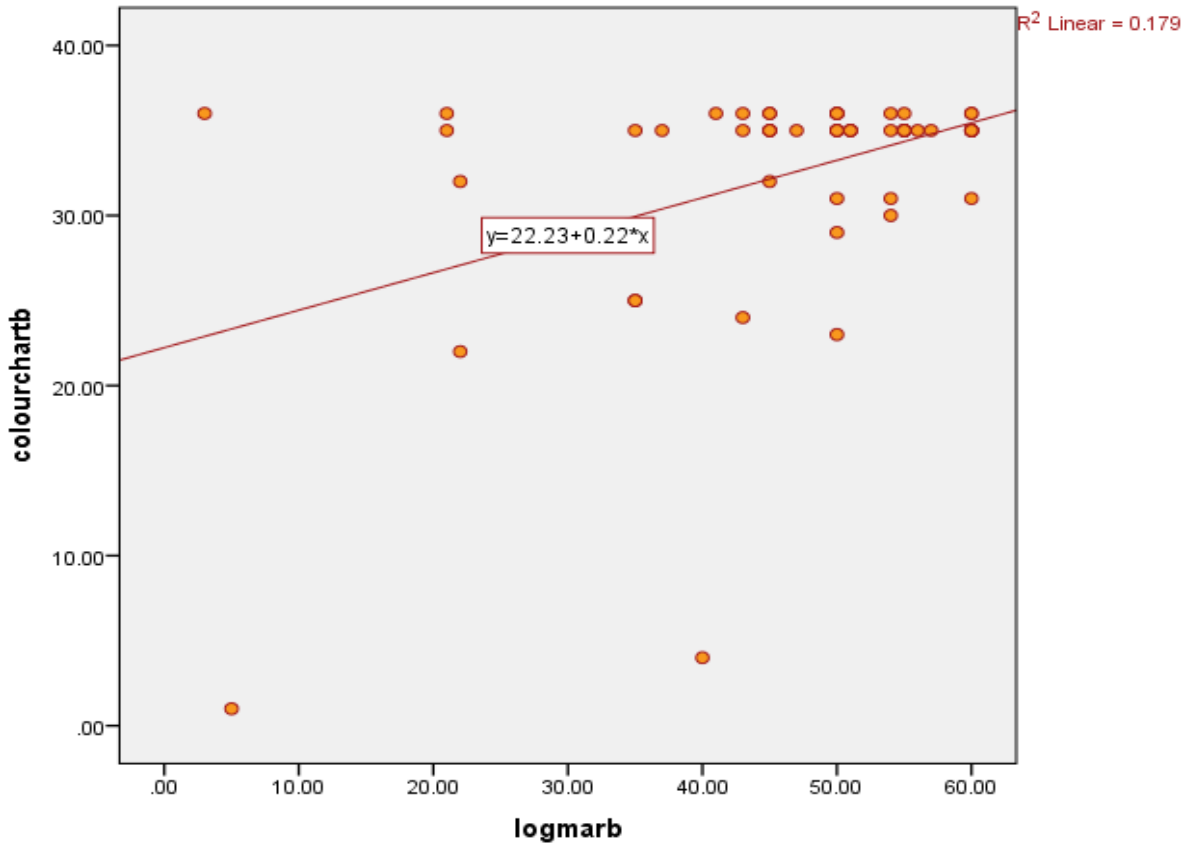


Figure 9.16: Scatter plot showing Sloan 2.5 and HRR colour chart (both eyes)

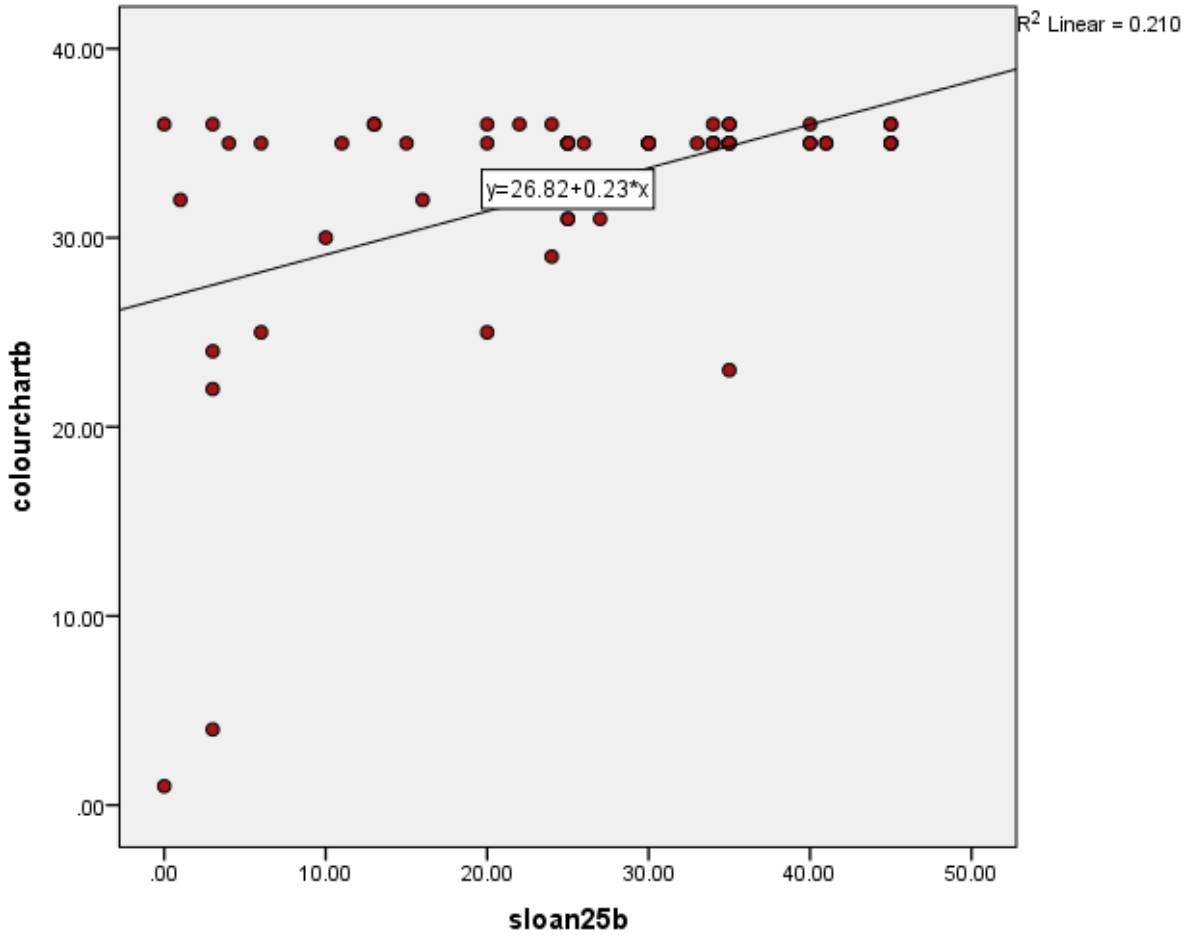


Figure 9.17: Scatter plot showing Sloan 1.25% and HRR

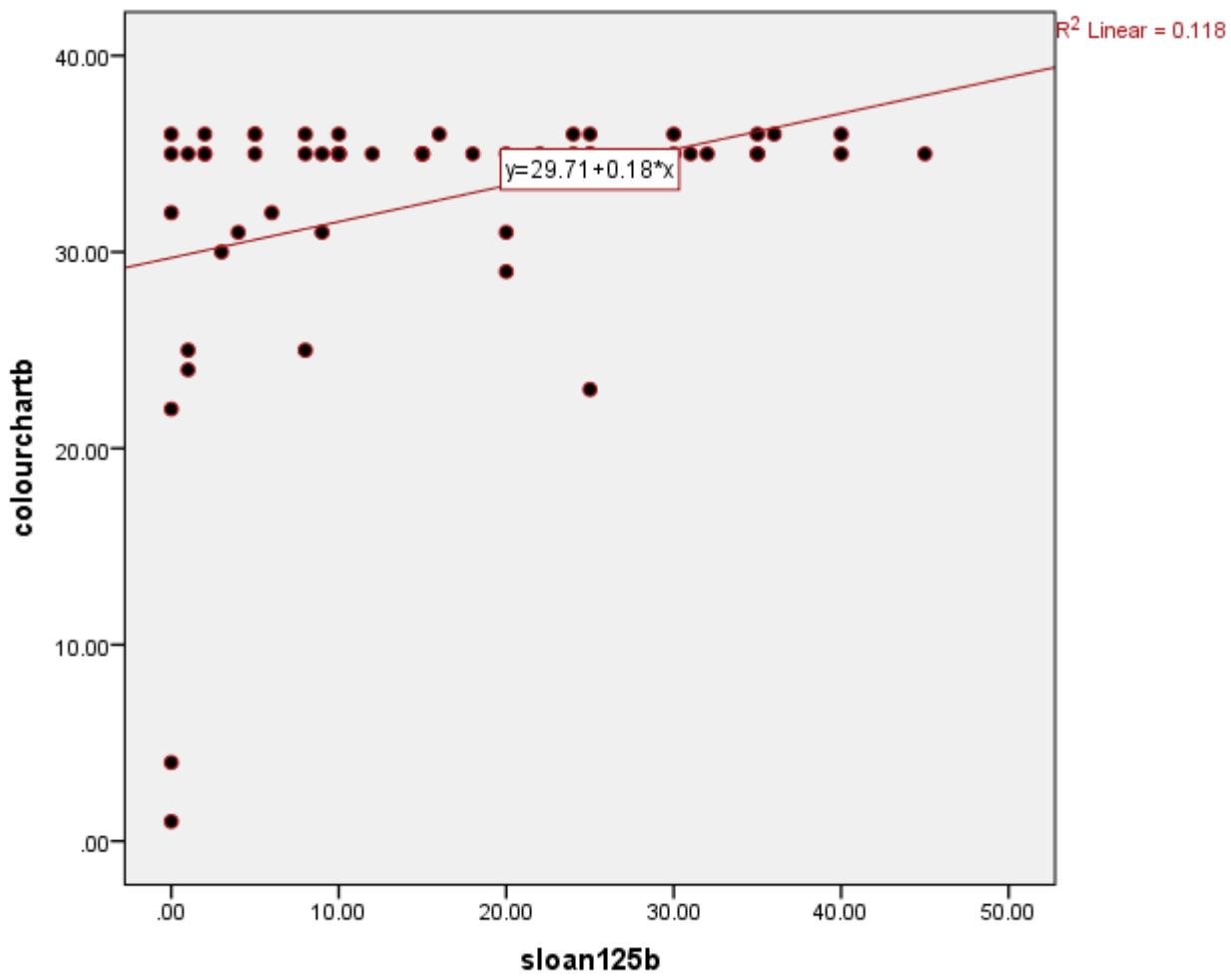


Table 9.18: Test for bi-variate correlation (Pearson) was then done on each of the 2 variables as shown below.

Correlations

BE= both eyes		Colour chart BE	Sloan125 BE	Sloan25 BE	Logmar BE
Colour Chart BE	Pearson Correlation	1	.344*	.458**	.423**
	Sig. (2-tailed)		.010	.000	.001
	N	55	55	55	55
Sloan125 BE	Pearson Correlation	.344*	1	.801**	.522**
	Sig. (2-tailed)	.010		.000	.000
	N	55	55	55	55
Sloan25 BE	Pearson Correlation	.458**	.801**	1	.658**
	Sig. (2-tailed)	.000	.000		.000
	N	55	55	55	55
Logmar BE	Pearson Correlation	.423**	.522**	.658**	1
	Sig. (2-tailed)	.001	.000	.000	
	N	55	55	55	55

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

The above table and scatter plot indicates a positive correlation between colour vision, LogMAR and Sloan charts.

The scatter plot though shows a positive correlation exhibiting strong proximity to the best-fit lines. There are few outliers, which generate negligible error in the interpretations.

The Pearson correlation and its significance in the above table help us make some conclusive assumptions. The correlation coefficient of each pair is very significant. Sloan 1.25% chart shows a significance of 1, which indicates a strong correlation between disturbance in colour vision and disturbance of

contrasts vision in disease-eyes due to damage of retinal ganglion cells (RGC) in MS. Color vision, reflects damage of retinal ganglion cells (RGC) and hence can serve as a marker of RGC

damage in MS.

Figure 9.19: Age at optic neuritis and deficiency of colour vision

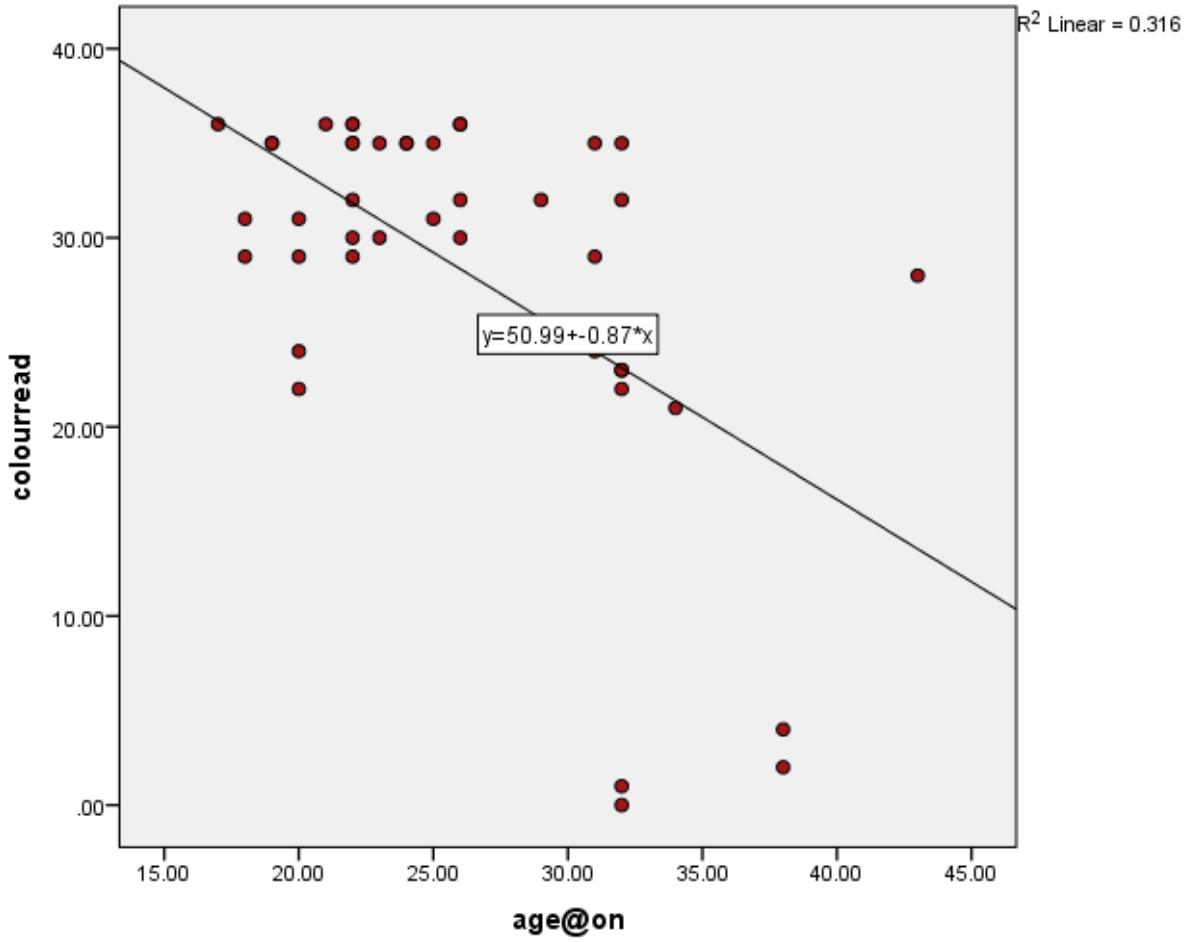


Table 9.20 : Correlations (significant at the 0.01 level (2 tailed)

		Colour read	age@on
Colour read	Pearson Correlation	1	-.562**
	Sig. (2-tailed)		.000
	N	44	44
Age@ on	Pearson Correlation	-.562**	1
	Sig. (2-tailed)	.000	
	N	44	44

9.7. Age at optic neuritis

The result of the number of colour chart read by patients in the optic neuritis group was explored. The data was split using the data function on SPSS.

Further a scatter plot was applied to this subgroup.

Interesting results revealed a significant negative correlation between the ability to read the test plates on the colour chart and age at optic neuritis.

Patients with early onset optic neuritis (age of 25 or less) had better colour vision compared to the late onset optic neuritis (>than 25years of age). This throws light on the mechanism of repair and macrophagic activity in early onset optic neuritis in MS.

This fact can be tested further by future researchers by conducted studies on a bigger sample.

Chapter 10

Result 3:

Evaluation of Humphrey's visual fields in disease and control

10.1. Humphrey's Visual field test

The Humphrey Visual Field is standard tests that allow us to look for defects in the sensitivity of the eye.

All patients and controls had Humphrey test performed. There were few occasions that the patients were fatigued and hence patients had to be retested to obtain a reliable field. Unreliable fields were discarded as no conclusion can be drawn from those fields. We did the 24-2, SITA Standard (Swedish Interactive Threshold Algorithm) testing protocol.

All patients were tested using the current prescription glasses. A automated focimetry was performed to calculate the refractive error which could then be incorporated into the testing system.

Patients had a good explanation about the procedure and few patients had undergone similar testing at the opticians in the community. There was no difficulty in obtaining reliable fields in all patients and controls though we had to repeat tests in 8patients to obtain consistent results. Out of the 8, 5 had a second testing and 3 had three testing done before obtaining acceptable fields.

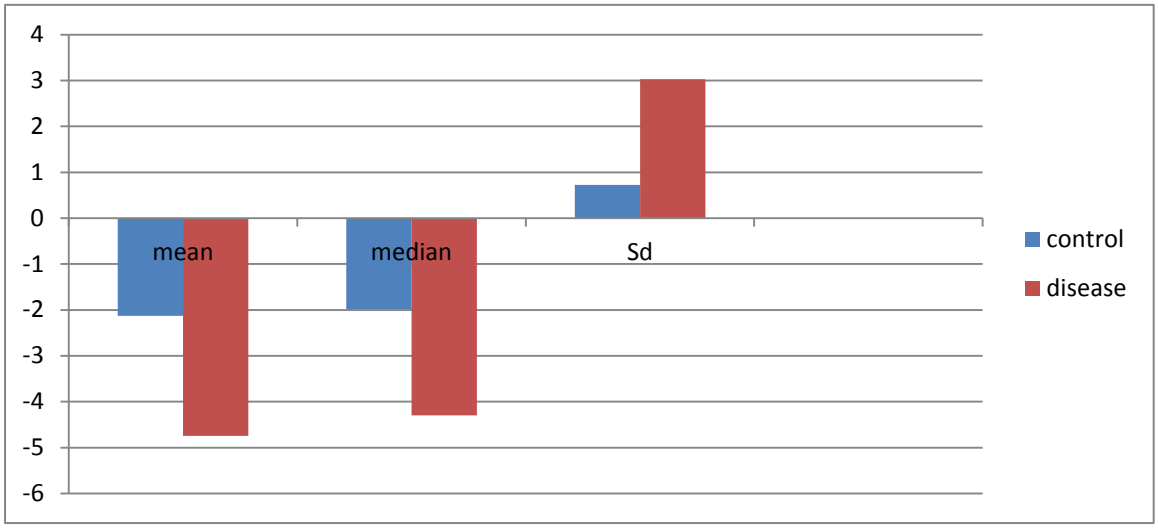
The parameters that were considered in the visual field tests are mentioned below:

1) Mean deviation
2) PSD
3) 8X8 square along the central fixation was analysed and correlated with posterior pole OCT.

Table 10.1: Mean deviation in controls and cases

Mean deviation in decibels	Controls	Disease	Optic neuritis group	Negative optic neuritis group
Mean	-2.13019	-6.74	-6.2	-5.9
Median	-1.98	-6.3	-6.1	-5.3
Standard deviation	0.728	3.025	2.01	3.2

Figure 10.2: Bar chart representing the data on mean deviation in control and cases



Pattern Standard deviation (PSD)

The study of PSD showed significant results. Though the loss of function was global there was a pattern to superior, inferior, nasal or temporal visual loss.

Table 10.3: Pattern standard deviation in controls and disease

PSD in dB	Controls	Disease	Optic neuritis group	Negative Optic neuritis group
Mean	-1.13	-5.74	-5.94	-3.2
Median	-1.36	-5.3	-5.5	-2.9
Standard deviation	0.92	2.92	1.1	0.6

8X8 square of Visual sensitivity

This specific parameter was tested and its correlation with RNFL thickness on posterior pole OCT analysis was explored. This correlation will be presented in the posterior pole OCT chapter.

Chapter 11

Result 4: Evaluation of parameters on OCT in disease and controls.

11.1. Optical Coherence Tomography in Multiple Sclerosis:

A structural assessment: A window into the mechanisms of Multiple Sclerosis

The pathophysiology of multiple sclerosis (MS) is characterized by demyelination, which culminates in a reduction in axonal transmission. Axonal and neuronal degeneration seem to be concomitant features of MS and are probably the pathological processes responsible for permanent disability in this disease. The retina is unique within the CNS in that it contains axons and glia but no myelin, and it is, therefore, an ideal structure within which to visualize the processes of neurodegeneration.

Optical coherence tomography (OCT) can provide high-resolution reconstructions of retinal anatomy in a rapid and reproducible fashion ideal for precisely modelling the disease process in MS.

Aim: To determine the relation between parameters on OCT and visual function tests.

11.2. Methodology

Structural assessment was done using with the Spectralis OCT and analysis was run on RNFL, GCIP, Macular volume, PPRNFL, Choroid and RNFL of posterior pole.

The structure was correlated with function of vision such as form, contrast, colour and visual field using LogMAR, Sloan , HRR charts along with parameters on visual fields.

11.3.Testing for coefficient of repeatability (variability)

We estimated coefficients of repeatability for Spectralis optical coherence tomography (OCT)-derived automated retinal thickness and volume measurements in 10 healthy patients and 10 patients with Multiple Sclerosis, not recruited into the study, though fulfilling the inclusion and exclusion criteria for the study.

The patients underwent four consecutive "fast" volume scans at a single session using one OCT device operated by one of two experienced operators. Bland-Altman coefficients of repeatability (CR) were calculated for automated retinal thickness measurements, center point thickness, total macular volume, RNFL, GCIP and PPRNFL. Scans were evaluated for significant automated retinal boundary detection error and revised estimates for CR calculated with these scans excluded.

Coefficient of reliability in controls and disease

CR for the macular volume was 1.03μm (95% confidence interval [CI] 0.89 - 1.35μm). The CR for RNFL and GCIP ranged from 2.54 to 3.25μm. The CR for PPRNFL was 4-6μm. Scan sets of subjects did not show any significant boundary detection error and centre recognition error.

Retinal thickness measurements in subjects with MS obtained using Spectralis OCT are considerably less variable and consistent with good coefficient of reliability and repeatability.

We used the Spectralis OCT, Heidelberg, Germany. The benefits of high resolution, eye tracking, reference setting, noise reduction and low coefficient of variability were the benefits of using the Spectralis model with auto segmentation technology. We had financial constraints to obtain the N-Site software to analyse the PPRNFL and hence we continued assessments using the existing software**.**

(The N-site Analytics™ software provides a unique analysis of the peripapillary RNFL. Based on the N-site normative database, a classification colour scheme indicates not only axonal loss but also oedematous changes).

Macular OCT allows us to image the retinal nerve fiber layer (RNFL) at the macula .In contrast ,the ONH OCT images the peripapillary RNFL, which

contains axons, the macula contains a large proportion of retinal ganglion cell neurons (about 34% of total macular volume). Hence study of different structure gives an overarching idea of the disease process in demyelinating optic neuropathy.

11.4. The parameters in the study are discussed in great details below.

1. Macular OCT RNFL

a) GCIP

b) Macular volume

2. Optic nerve OCT

c) PPRNFL

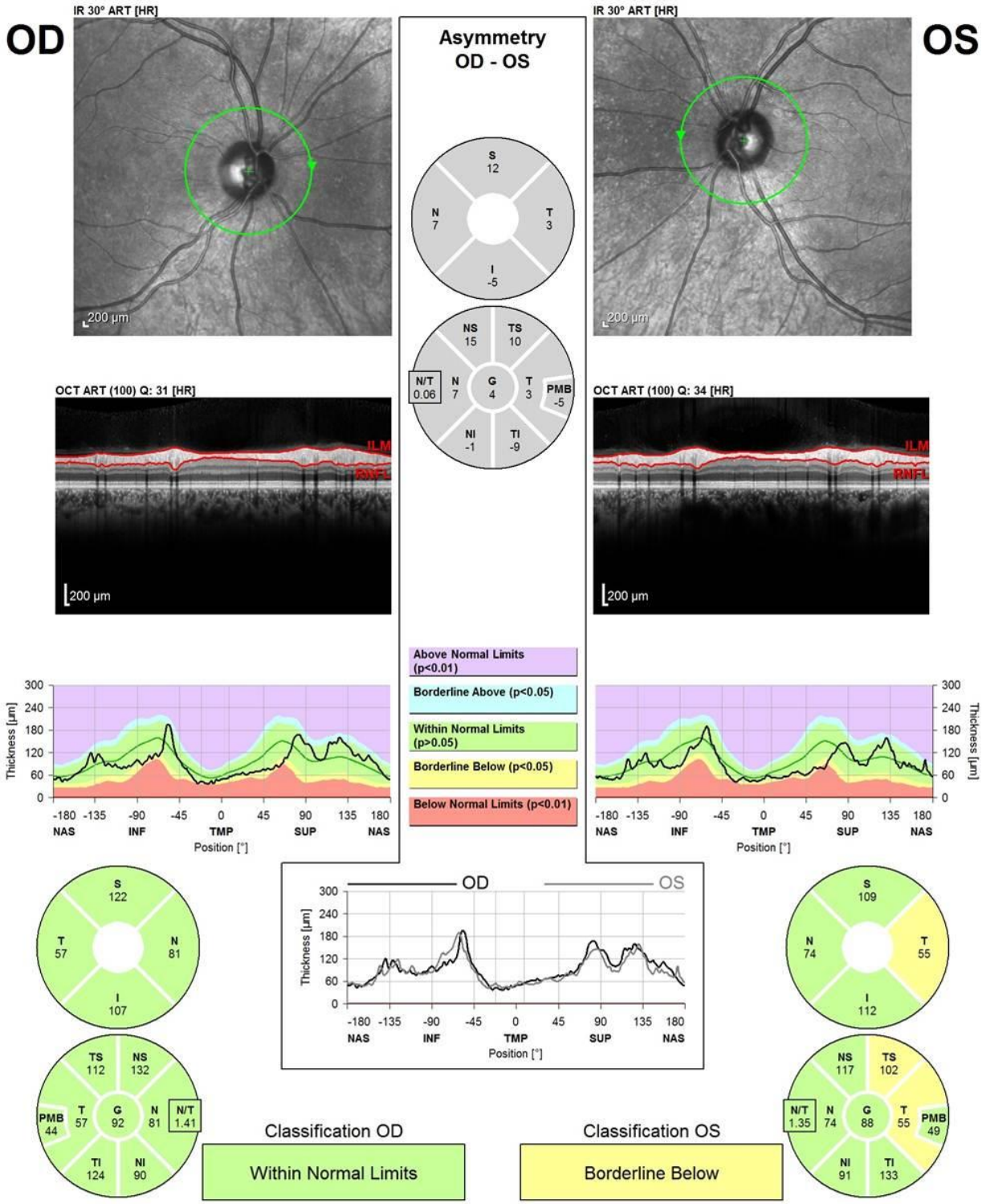
3. EDI OCT

d) Choroid

4. Posterior pole OCT

e) Asymmetry analysis

Figure 11.1: OCT of Optic nerve head (ONH)



Top image: The green line indicates the location of where the OCT image is taken around the Optic Nerve Head (ONH)

Second Image: The OCT scan is automatically segmented between the two red lines (the internal limiting membrane to the Retinal Nerve Fiber Layer (RNFL))

Third Image: The black line indicates the RNFL thickness for this patient. Colour coding is as indicated between the two graphs.

Bottom: Pie charts indicate sector thickness in the nasal, temporal, superior and inferior areas around the ONH, indicating borderline thinning in the temporal segment in the left eye.

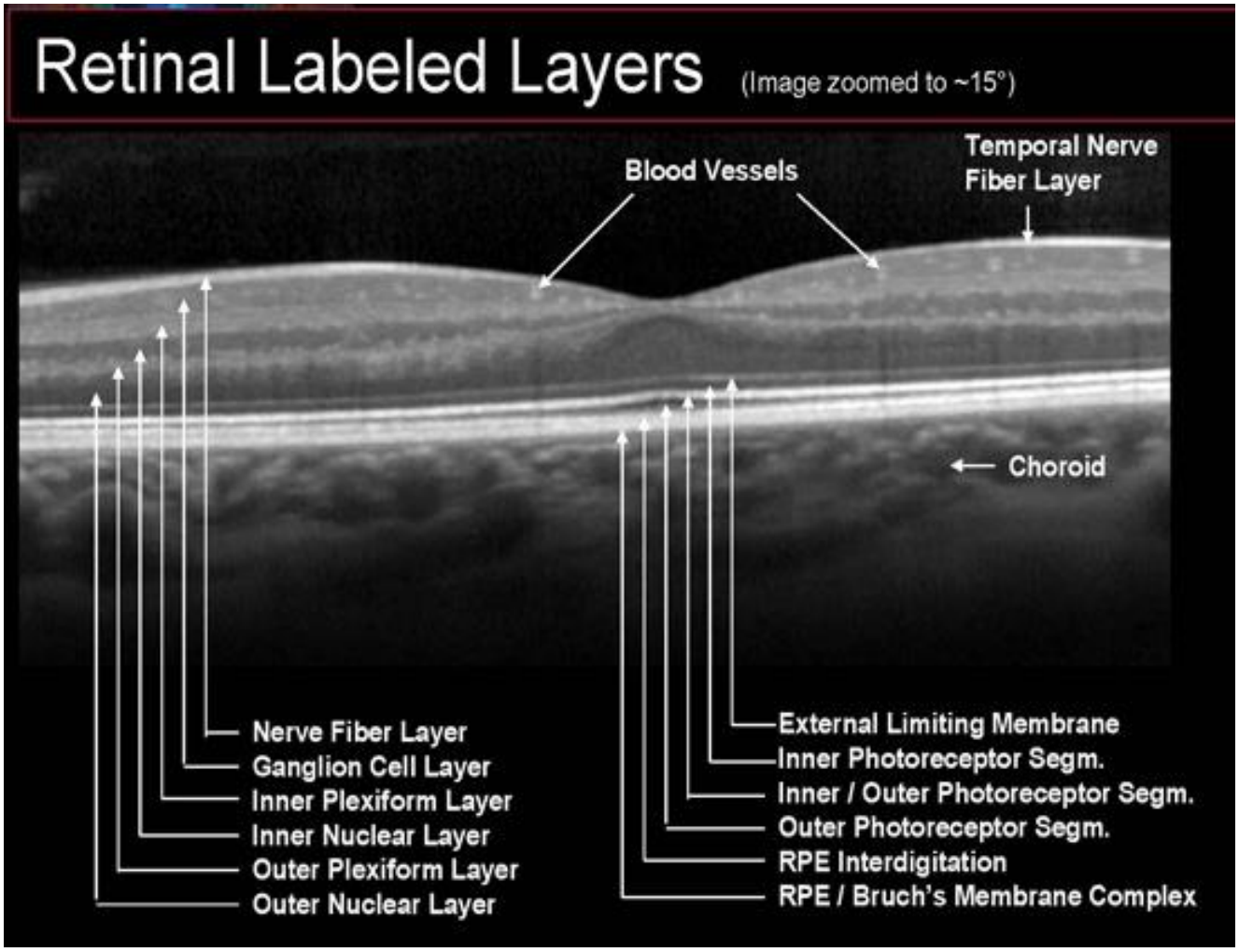


Figure 11.2: Normal OCT scan of macula

Figure 11.3: Posterior pole OCT showing the 64 grid macular and asymmetry analysis.

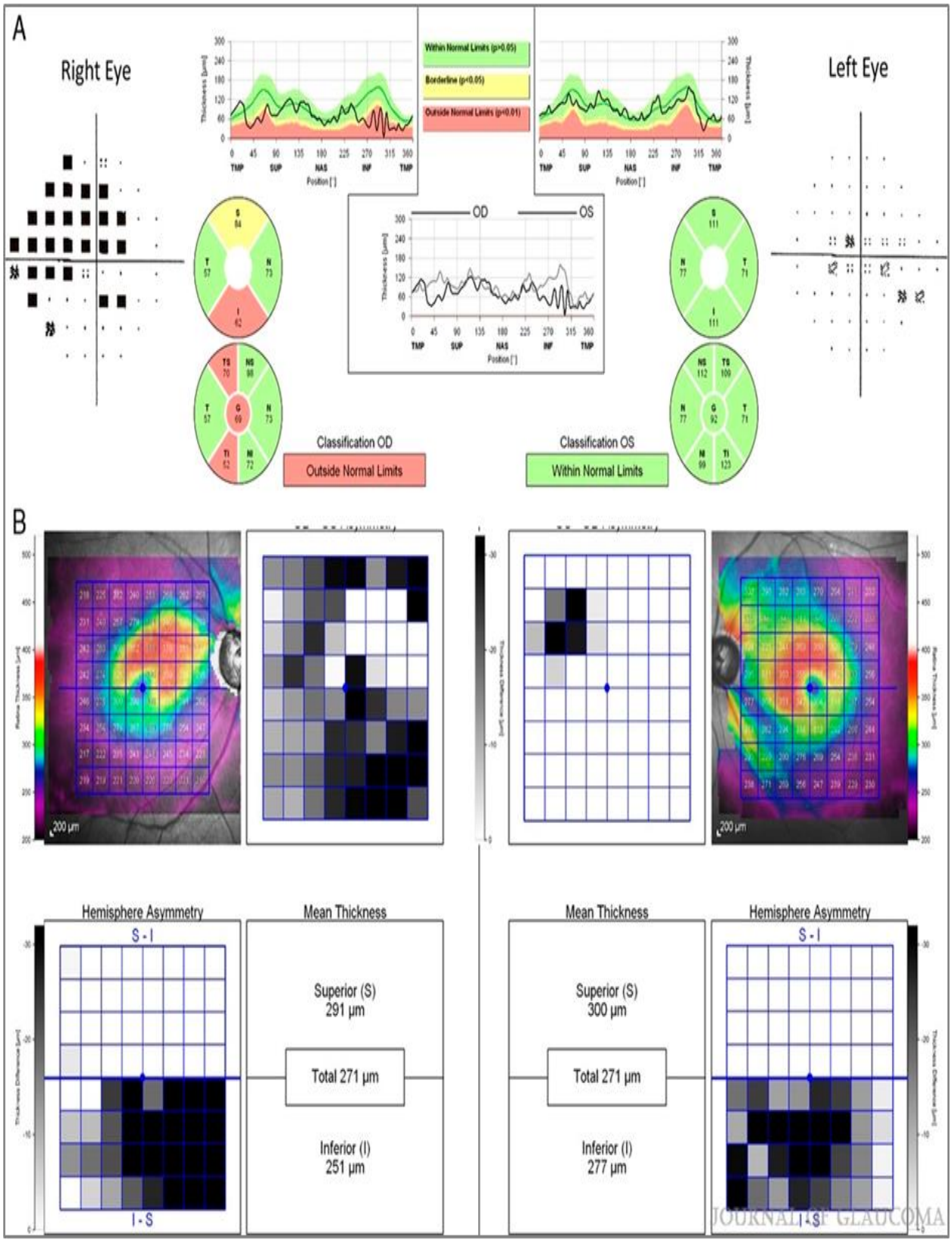
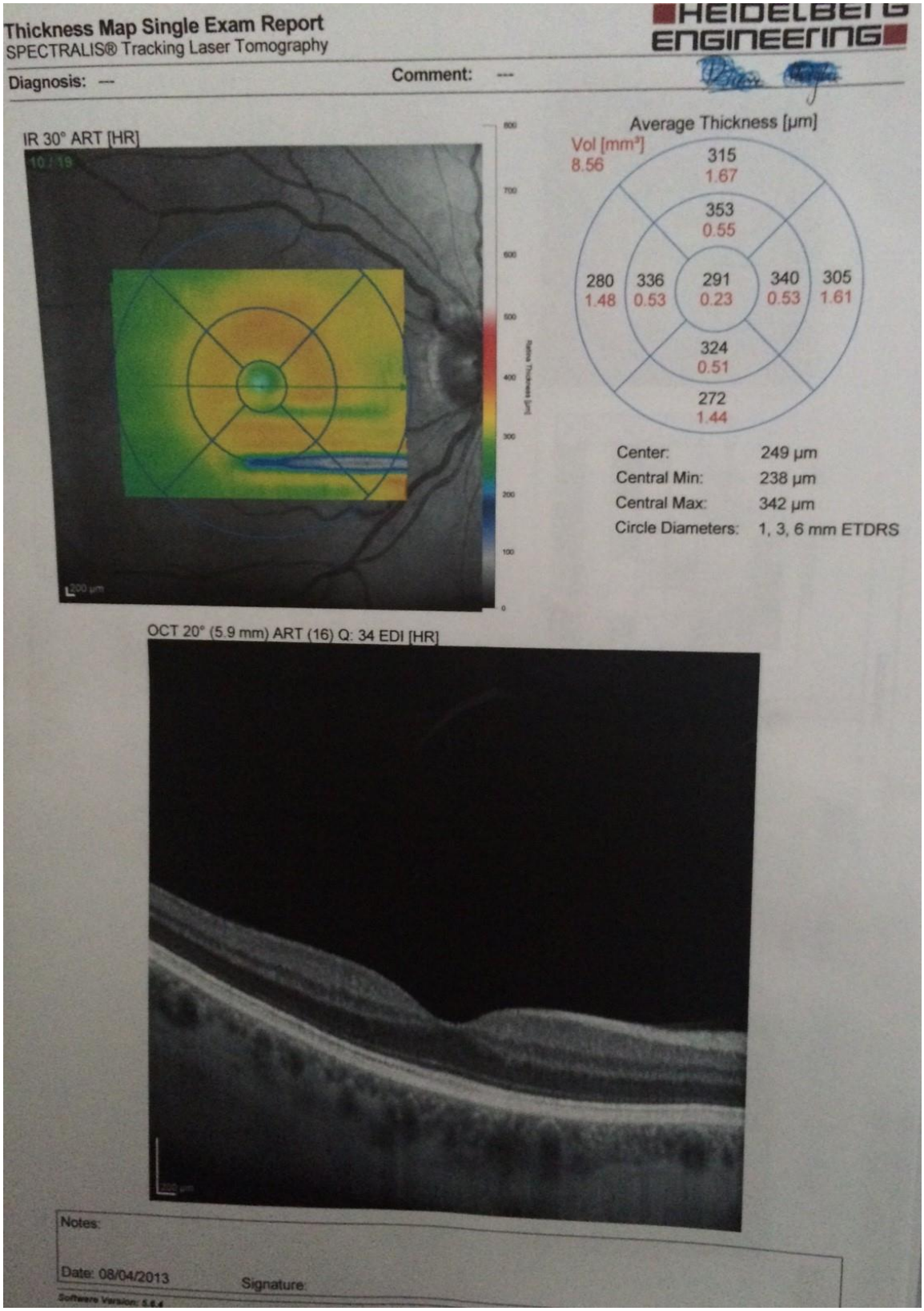


Figure 11.4: Single Exam report of Macular thickness map



11.5. OCT parameters in cases and controls

We studied the cases and controls for OCT parameters such as:

1) Retinal nerve fibre layer thickness (RNFL)
2) Ganglion cell –inner plexiform complex (GCIP)
3) Macular volume (MV)
4) Peripapillary RNFL (PPRNFL)
5) Posterior pole OCT (PPOCT)
6) Enhanced depth imaging OCT (EDI-OCT)

Table 11.5: Macular OCT parameters in Cases

N=110	Macular Volume mm cube	Macular RNFL Thickness microns	Ganglion cell-Inner plexiform Thickness microns	Peripapillary RNFL microns
Mean	7.935	23.99	82.43	85.91
Median	7.9	25.185	83.5	86
Standard Deviation	0.8351	5.26	8.86	15.49

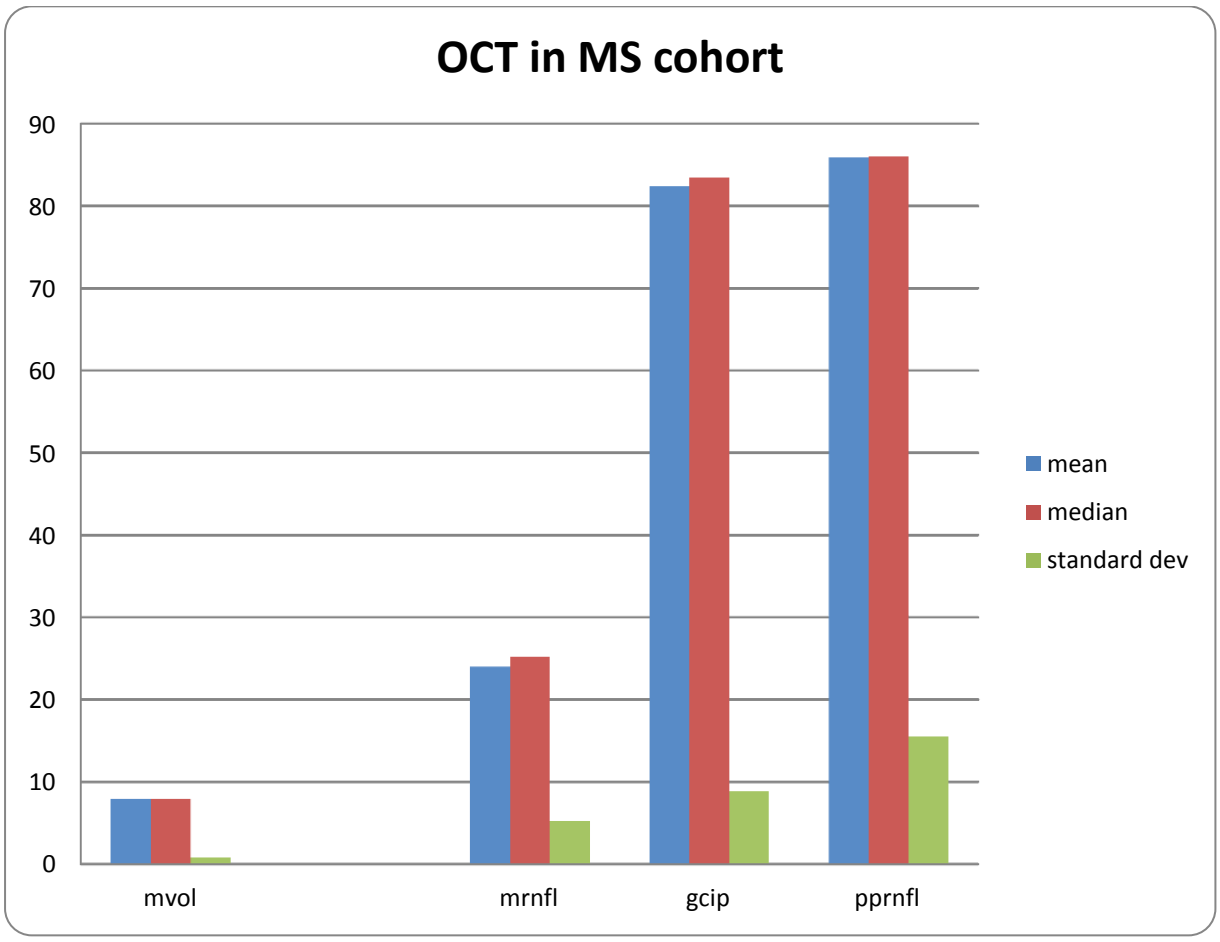


Figure 11.6:Bar chart showing the OCT parameters in Cases.

The disease group had a mean macular volume of 7.935 (cubic mm) with a standard deviation of 0.835.

The mean macular RNFL was 23.99 microns with a standard deviation of 5.26 microns.

The mean GCIP was 82.43 microns with a standard deviation of 8.86 microns.

The PPRNFL was 85.91 microns with a standard deviation of 15.49 micron.

Table 11.7: OCT parameters in Controls

N=50	Macular Volume mm cube	Macular RNFL Thickness microns	Ganglion cell-Inner plexiform Thickness microns	Peripapillary RNFL Microns
Mean	9.2	29.6	88.9	92.76
Median	8.91	29	88.6	91.76
Standard Deviation	1.76	6.12	8.87	10.24

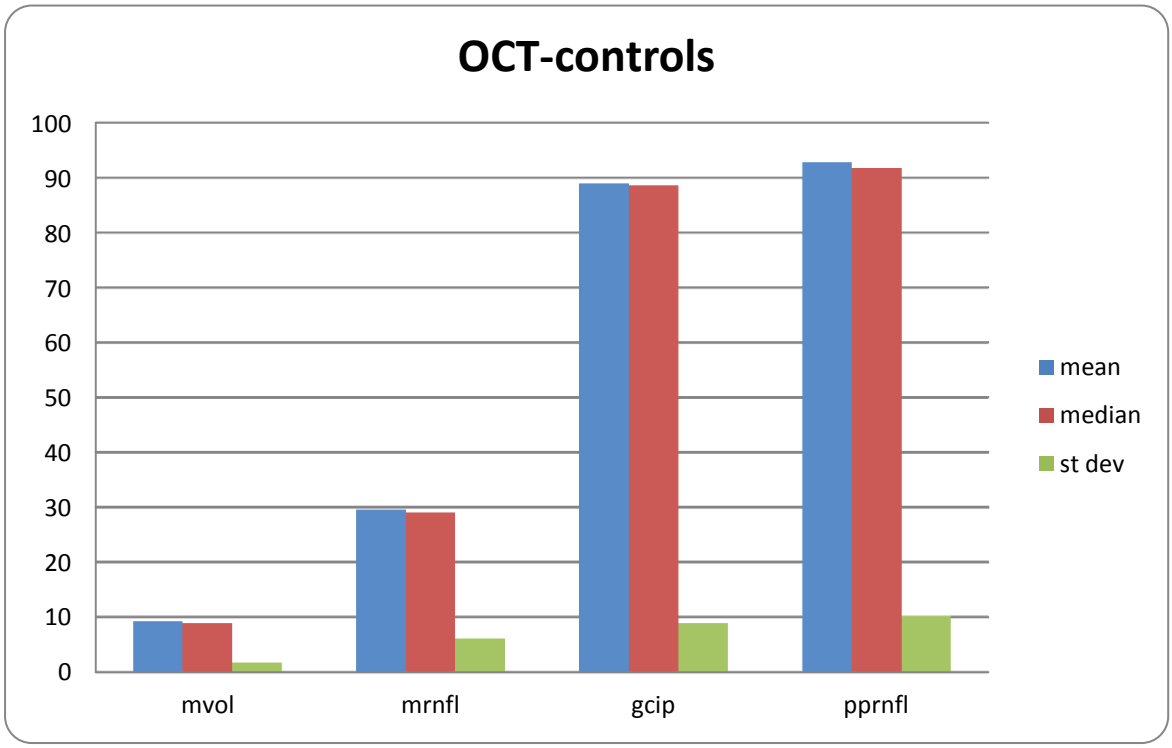


Figure 11.8: Bar chart showing the OCT parameters in Controls.

The control group had a mean macular volume of 9.2 (cubic mm) with a standard deviation of 1.7.

The mean macular RNFL was 29.6 microns with a standard deviation of 6.12 microns.

The mean GCIP was 88.9 microns with a standard deviation of 8.87 microns.

The PPRNFL was 92.76 microns with a standard deviation of 10.24 microns.

Table 11.9: Comparative data control versus cases

	Control	Disease
Mean macular volume (cubic mm)	9.2	7.9
Mean Macular RNFL (microns)	29.6	23.99
Mean GCIP (microns)	88.9	82.43
Mean PPRNFL (microns)	92.76	85.91

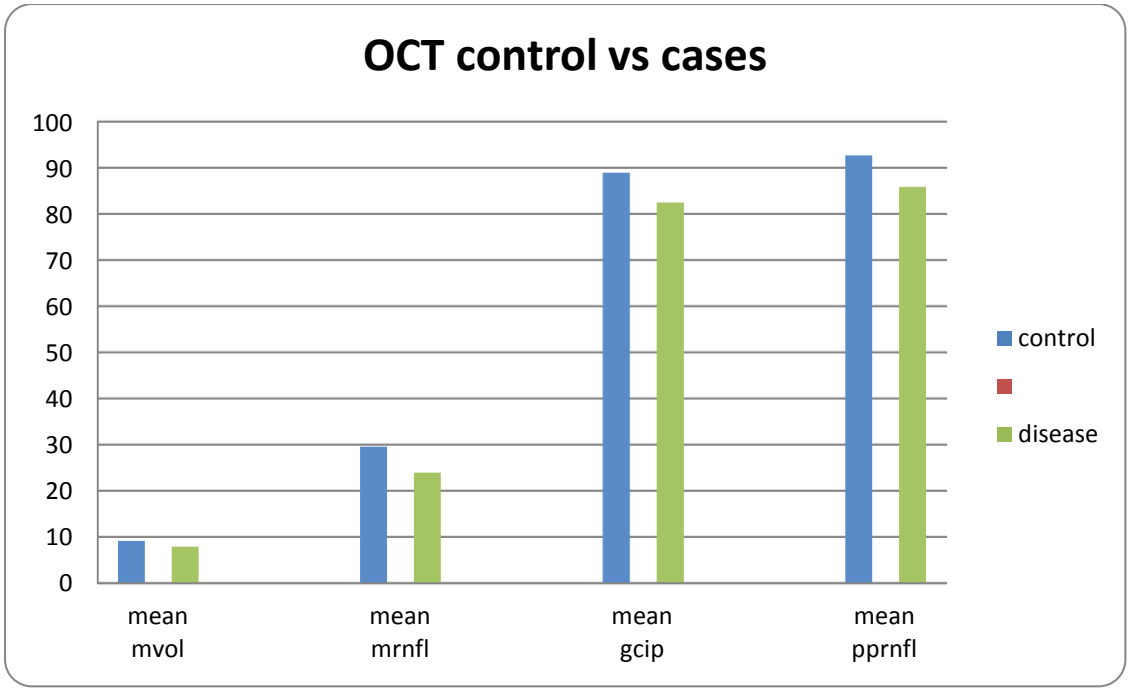


Figure 11.10 :Bar chart showing the comparision of OCT parameters in Controls versus cases.

The cases were further split into the ones who had optic neuritis. Analysis was redone in the subgroup. (Table 11.1)

Table 11.1	Controls N=	All Cases N=	Cases with history of optic neuritis N=	Cases with no history of optic neuritis
Mean macular volume	9.2	7.9	6.43	8.1
Mean macular RNFL	29.6	23.99	21.65	24.98
Mean GCIP	88.9	82.43	79.34	84.00
Mean PPRNFL	92.76	85.91	79.75	86.91

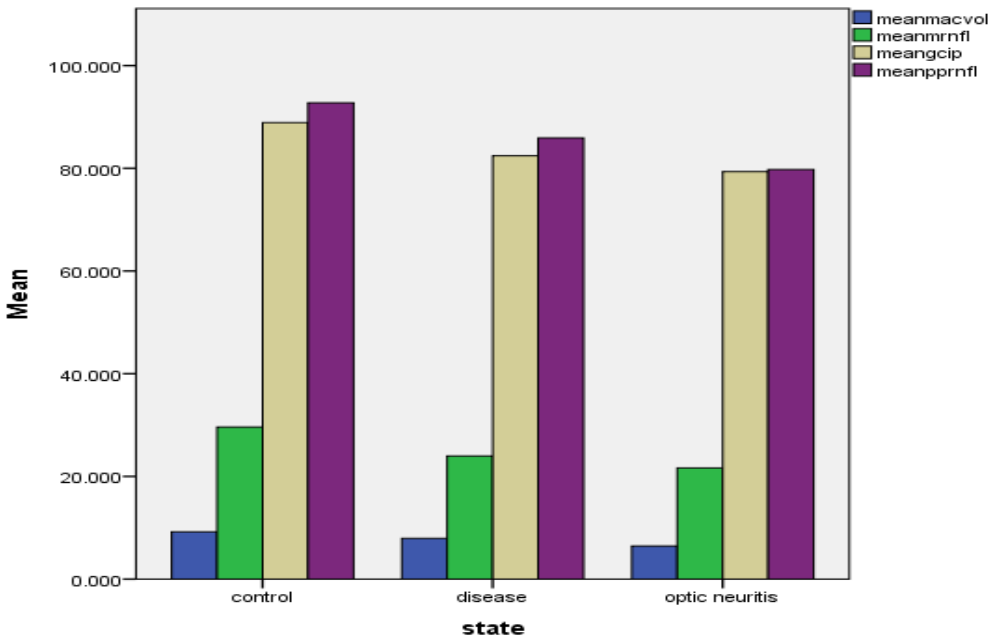


Table 11.1 and figure 11.2 : Comparative data of control , cases and those with optic neuritis

11.6. Conclusion

The thickness of the macular RNFL was reduced by 27% in the affected eyes of the patients with MS versus the control eyes (P<0.01), and by 8% when affected eyes were compared with the 'unaffected' eyes of the same patient (P<0.01). Even in the clinically unaffected eyes of patients, however, there was a 13% reduction in RNFL thickness when compared with control eyes (P<0.01).

The macular volume (a reflection of retinal ganglion cell neuronal integrity) was reduced by 31% in the eyes of patients with a history of ON when compared with control eyes (P<0.001), and by 17% in the affected versus the unaffected eye of the same patient (P<0.001).

Patients with MS who have ON sustain 10–40 µm of RNFL loss within a period of approximately 3–6 months. This finding is striking given that the RNFL is only about 110–120 µm thick by the age of 15 years, and that most normal will lose only about 0.017% per year in retinal thickness, which equates to a decrease of approximately 10–20 µm over 60 years.

The patients have to be followed for few years to identifying an injury threshold within the RNFL and thinning of the RNFL that corresponds to decline in visual function, as measured by contrast charts and automated perimetry.

This does call for a prospective study in future for a minimum duration of 5 years to be conducted in such patients to determine the threshold of the RNFL injury at which visual loss becomes evident. This will generate a patient specific RNFL threshold instead of a blanket approach to RNFL loss in MS.

Macular volume and macular RNFL show a strong correlation in disease. A drop in 14% of the macular volume in cases correlated with a drop in macular RNFL by 19% .The eyes with Optic neuritis showed more profound correlation with a drop in macular volume by 31% and 27% drop in macular RNFL.

Macular RNFL seems to be a sensitive parameter determining change in threshold vision. GCIP is more sensitive to changes in patients with positive history of ON.

Thickness in millimeters	Cases (p values)	Controls	Optic neuritis group	Treatment group
Macular volume	7.9 (0.001)	9.2	6.43	8.1
Macular RNFL	23.99 (0.01)	29.6	21.65	25.3
GCIP	82.43 (0.0001)	88.9	79.34	85.2

Table 11.3: Comparative data of OCT parameters in cases, controls, optic neuritis and treatment group

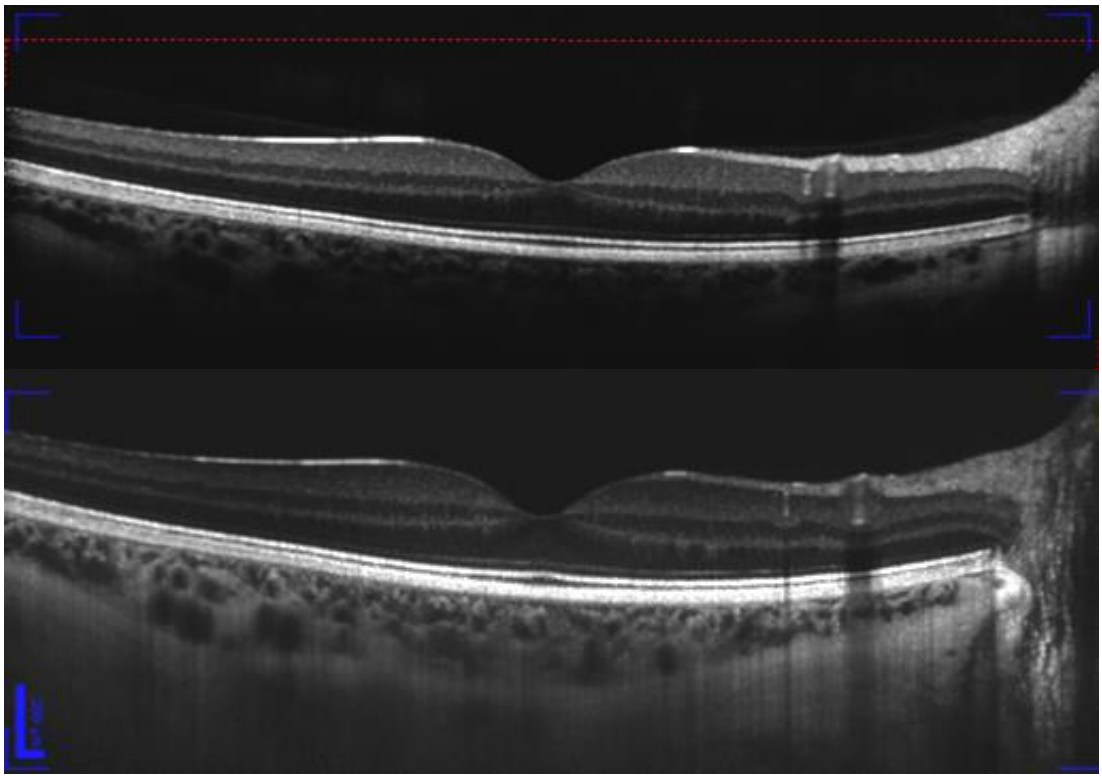
Chapter 12

Result 5: Enhanced depth imaging (EDI-OCT) for the choroid

Enhanced Depth Imaging OCT (EDI)

EDI-OCT is a new imaging modality to enable high-resolution OCT imaging of the choroid along with retinal layers and lamina cribrosa. It allows detecting structural changes ‘beyond the retinal pigment epithelium’ in a reproducible fashion difficult to capture with standard OCT**.** This function is unique to the Spectralis model of OCT that we used in our study. During a single examination, it can be easily switched between ‘normal’ and ‘EDI-OCT’ mode making it friendly for both the patient and the operator.

Figure 12.1: Image below showing the EDI function in use on the Spectralis model of OCT. Note the difference from the upper image with no clear definition of choroid and sclera .



12.1. Aim and methodology

All patients and controls underwent EDI OCT. A total of 110 scans were obtained from the disease and 50 scans obtained from the controls.

The main aim for using the EDI OCT:

1) To explore the thickness of the choroid in patients with Multiple Sclerosis, compare it to the controls. Multiple sclerosis is an autoimmune condition which results from a breakdown of the blood brain and blood ocular barrier. The Retinal pigment epithelium (RPE) forms the outer ocular barrier and separates the retina from the vascular choroid. There are no studies until date that has looked at the choroid in MS and control.

2) Embryologically the choroid layer along with ciliary body is similar to the choroidal plexus in the brain. Choroid plexus secrete the cerebrospinal fluid and oligoclonal bands have been detected in patients with MS in the CSF. Choroid is the main vascular bed and Bruch's membrane contributes to the retinal barrier. Though it is challenging to study the composition of the blood in the choroid, it is not impossible to study its structure. The structure of choroid may give an indirect clue to the function of the vascular system. The recent introduction of EDI OCT has made it possible to study the structure of the choroid in great detail.

12.2. Results

We studied the thickness of the choroid in both cases and controls. The spread of data is represented in the table (12.2) below.

EDI	Mean microns	Median microns	SD microns	First quartile	Third quartile
Controls	261	267	13	265	287
Cases	263	267	71	236	289

Table 12.2: EDI OCT in controls and disease

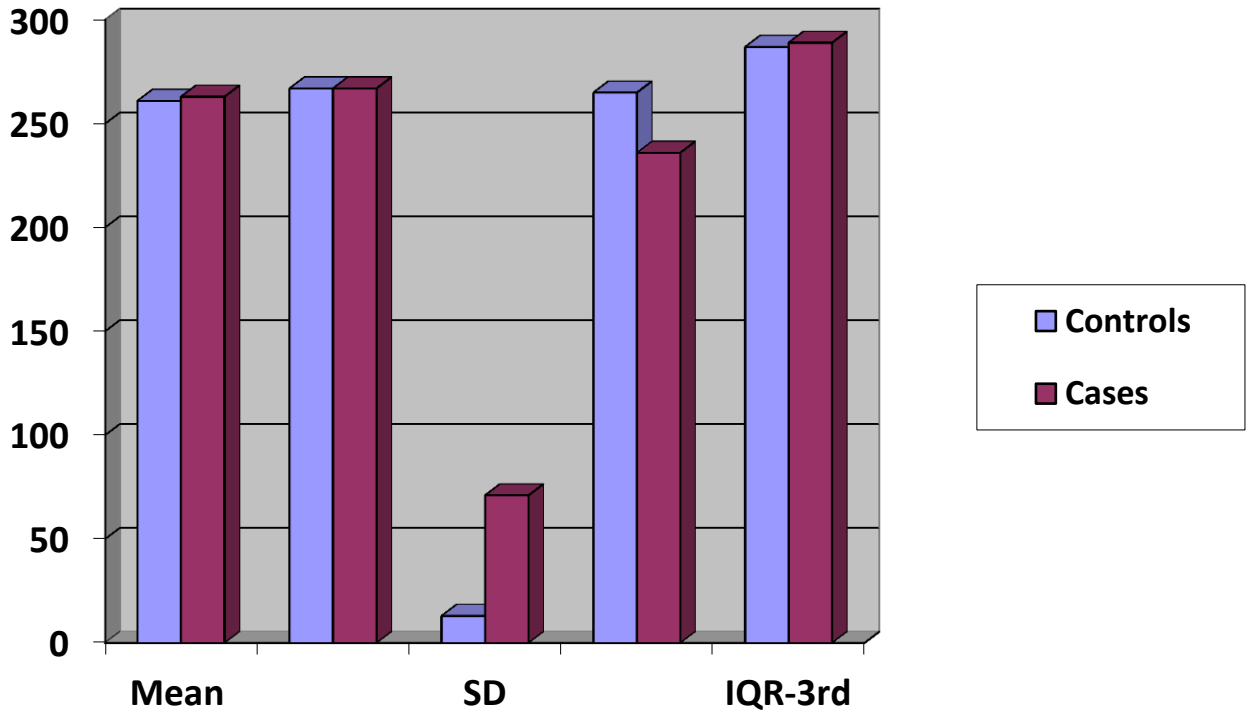


Figure 12.3: EDI OCT in controls and disease

The cases were further studied in the range of thickness they exhibited to see if the groups were any different.

Table 12.4: Grouping of cases based on choroid thickness

EDI (thickness)	200-316	>316	<200
Cases	85	12	13
Thickness	Normal	Thick	Thin

The EDI was studied in the 3 sub-groups and a sub group analysis was done.

1) EDI (200-316 microns)

Patients with multiple sclerosis seem to have a greater SD of 71 compared to that of 13 in the control group. The group had variable ocular parameters and a correlation was established with the LogMAR, Sloan Chart and color charts. Correlation was also carried out on the retinal parameters using OCT such as mean RNFL and GCIP.

2) EDI (> 316microns) Mean 332 and Standard deviation 18

Patients in this group either had history of optic neuritis, poor letter scores on Sloan2.5%, Sloan 1.25% charts and color charts with progression of EDSS with time and were treated with disease modifying agents. Overall, this group represented the active MS group.

3) EDI (< 200 microns) Mean 157 and SD 17

Patients in this group had a history of severe optic neuritis with reduced visual function after the episode. Patients were treated with 3-5 course of intravenous steroid during the span of the disease process. They had poor letter scores on Sloan's chart especially 1.25% and had variable relation to EDSS.

12.3. Conclusion:

EDI- OCT forms an important investigative modality in the autoimmune related neuro-degenerative process in Multiple Sclerosis. The process of neurodegeneration is reflected by the thinning of the retinal nerve fiber layer in chronic cases while the autoimmune activity is reflected by the thickness of the choroid.

Thicker choroid in Multiple sclerosis may indicate an active immune process and can form an important adjunct to neurological assessment, such as EDSS and MSFC. EDI OCT can most probably be used to detect response to disease modifying treatment and can form a prognostic indicator. This study has to be extended to a bigger cohort of patients to confirm the findings of this preliminary study.

Thinner choroid indicates a moderate to severe global neurodegenerative process as shown by suppressed ocular and neurological function. Steroids are commonly used immunosuppresant during a period of relapse. The use of steroid during a relapse is debatable, as it is known to accelerate the neuro-degenerative process of global neurons on a long-term follow up. There are no

guidelines on patient selection for use of steroids, neither is there a way of monitoring response to treatment after a steroid pulse treatment.

As ophthalmologist, we use the term steroid responders in those who intraocular pressure raises after treatment with topical steroids. EDI-OCT may help in identifying patients and sub-grouping them into steroid responders and non-steroid responders. It may help in patient selection and setting up a guideline for use of steroid in this complex disease.

In short, it will help identify patients who will overweigh the benefits in contrast to risk of treatment with steroid therapy.

Chapter 13

Results 6: Posterior pole OCT in cases and controls

Posterior pole OCT

The Posterior pole OCT is one of the common OCT protocol used in Ophthalmology for patient with suspicion of glaucomatous neuropathy. It mainly analyses the RNFL at the optic disc and peri-papillary zone along with assessment of the RNFL in the 8X8 mm zone centered on the fovea, aligned on the disc.

13.1. Aim of the study

1) To provide a quantitative comparison of the retinal thickness in the macula and circum -papillary retinal nerve fiber layer (RNFL) defects, in Multiple sclerosis and controls.
2) To explore and detect if posterior pole asymmetry can be characterized to optic neuropathy in MS
3) To correlate macular thickness on posterior pole OCT with Humphrey visual field (HVF) parameters

13.2. Patients and methods:

The patients and controls in the study underwent bilateral SD-OCT (Spectralis, Heidelberg Engineering,) using the GDA (glaucoma diagnostic) scan protocol. The protocol includes measurement of the RNFL in the optic nerve, circumferential peri-papillary area and posterior pole macular thickness analysis protocol. Briefly, the macular thickness analysis measures retinal thickness

along 64 squares in the central 20 degrees of each eye. The area is divided into an 8×8 mm grid, centered on the foveal pit and aligned with the optic disc, and consisting of small 3 degree by 3 degree squares, with the mean retinal thickness of each small square displayed. In addition, the average total retinal thickness, as well as the average retinal thickness for each macular superior-half and inferior-half, are calculated and displayed for each eye. The RNFL measurement is displayed as an average thickness for the quadrants as well as with the superior and inferior quadrants divided into nasal and temporal sections each.

Figure 13.1, shows an example of posterior pole OCT data collected from one of the study MS patient.

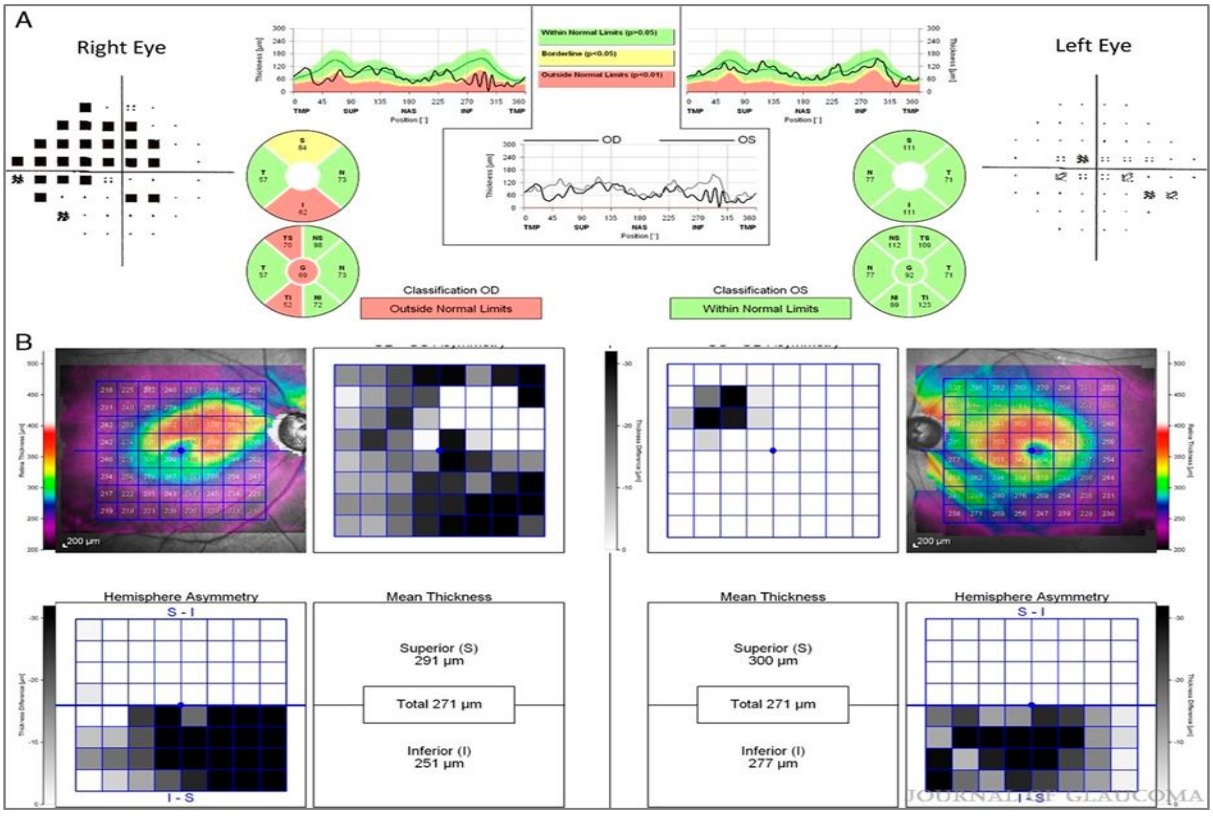
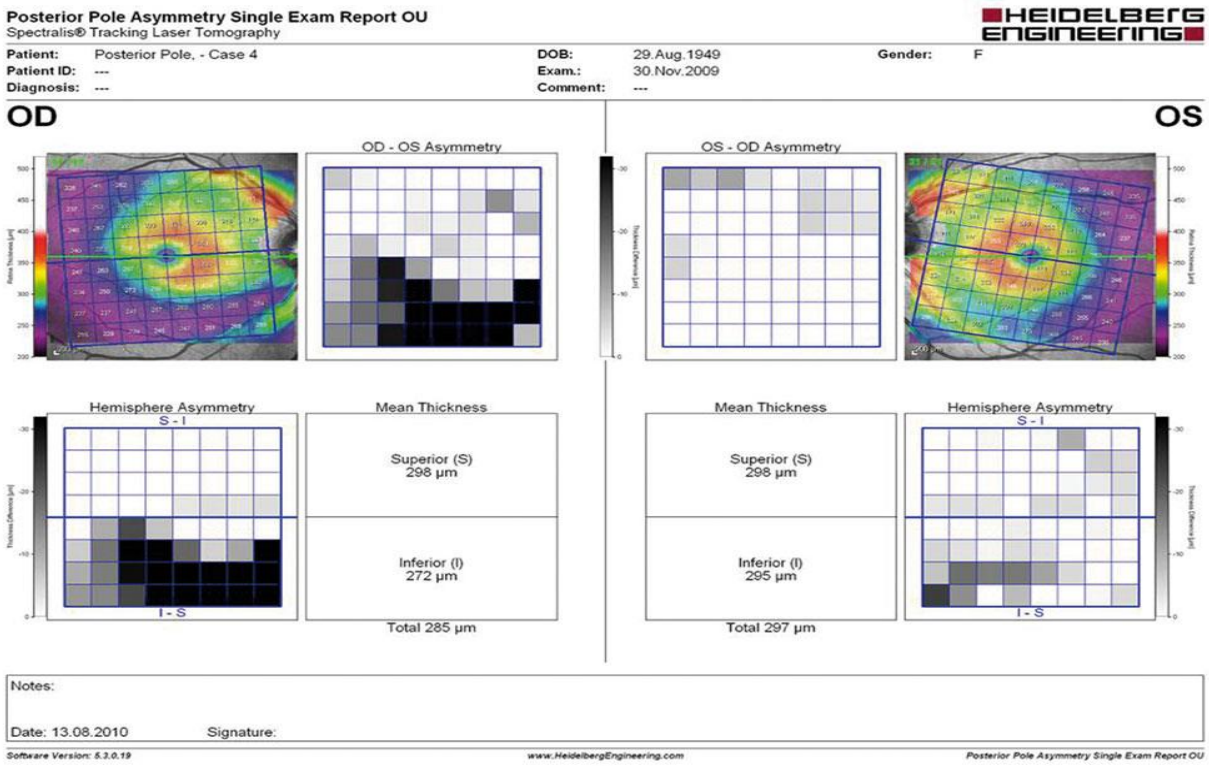


Figure 13.2, Spectral domain optical coherence tomography (SD-OCT) with asymmetry analysis.

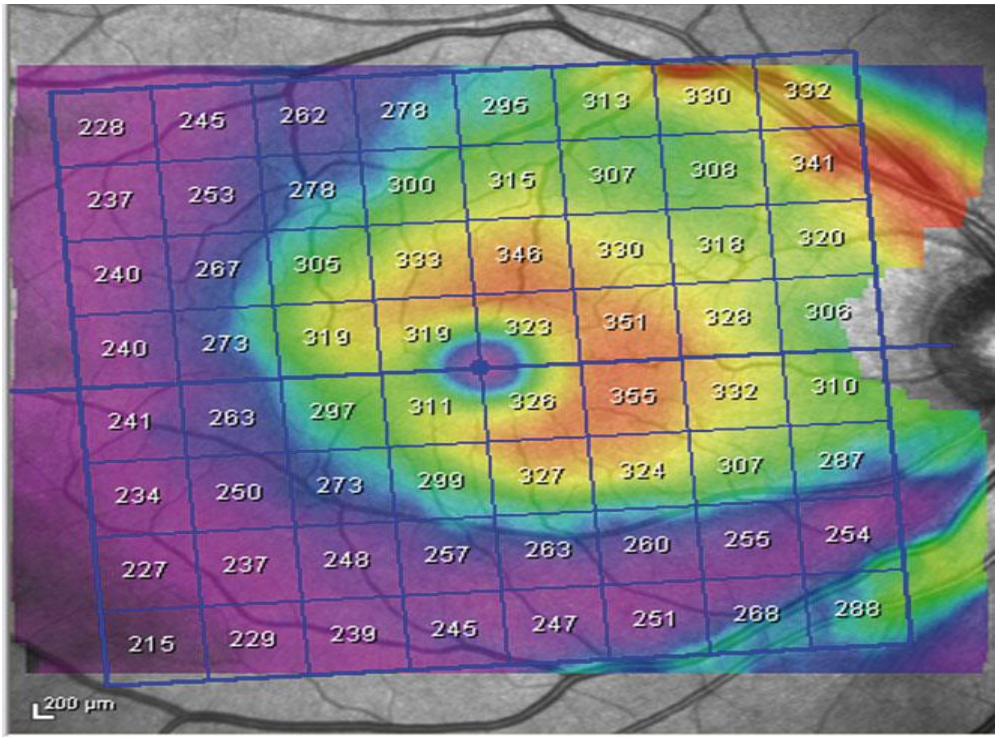


A. Visual field and SD-OCT analysis of the retinal nerve fiber layer (RNFL). The pattern standard deviation plots show a dense superior altitudinal defect and an inferior nasal defect in the right eye, with corresponding RNFL thinning inferiorly more than superiorly. In the left eye, the visual field shows some non-specific central and nasal areas of decreased sensitivity. The RNFL average thickness by quadrant is normal; however, the RNFL tracing shows a dip infero-temporally.

B. Asymmetry analysis of the posterior pole. The retinal thickness map overlays the infrared photo of the posterior pole for each eye. Inferior thinning

is noted in both eyes, seen quite clearly in the colour map; in addition, the RNFL defect noted in the left eye in (A) is seen to extend well into the macula.

Figure 13.3 showing point to point nalysis on posterior pole oct



The asymmetry analyses are displayed as gray-scale maps, with respective thinning shown progressively darker (30µm difference appears black, 20µm difference gray, and no difference or thickening appearing white). The asymmetry analysis comparing the right and left eyes (located between the maps) shows significant global thinning of the right eye compared with the left. The asymmetry analysis comparing the superior and inferior hemifield as well as the average retinal thickness measurements (total, as well as superior and inferior averages) are shown below the maps.

13.3. Results:

55 MS patients and 25 controls had OCT testing for Posterior pole analysis. All patients had visual field test carried out by Humphrey's field analyser.

Retinal thickness in the macula and peri -papillary area, in Multiple sclerosis and controls.

The peripapillary RNFL was analysed in disease and controls. The disease group showed an 8% decrease in the nerve fibre layer thickness compared to the control group and a 15% decrease in the peripapillary RNFL in the optic neuritis group. There was a 7% difference in RNFL between the optic neuritis group versus the no optic neuritis group. There was marked dip in the temporal RNFL of the optic disc in cases with and without optic neuritis. The loss of temporal fibers accounted for 25% drop in RNFL when compared to controls and 32% drop in RNFL in optic neuritis group.

Table 13.4: PPRNFL in control, disease and optic neuritis group

Mean thickness	controls	disease	Optic neuritis group
PPRNFL	92.76	85.91	79.75

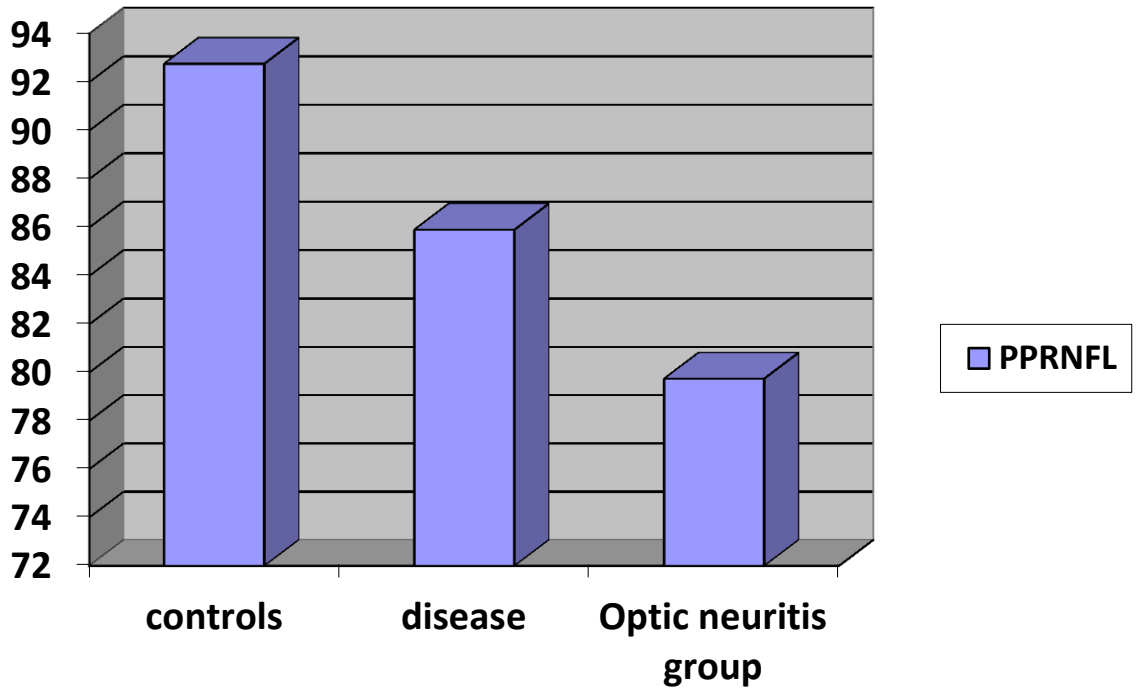


Figure 13.5: Graph showing PPRNFL in control, disease and optic neuritis group.

Table 13.6: Comparison of superior , inferior , temporal and nasal fibres.

PPRNFL	Controls	Disease	Optic neuritis group	DMT group
Temporal	54	49	33	47
Nasal	63	59	49	63
Inferior	102	78	69	72
Superior	98.8	83	77.8	82

Correlation of total macular thickness on posterior pole OCT with global measure of visual field were analysed.

Posterior pole OCT parameters were correlated with Visual field indices. Every square in the posterior pole OCT subtends an angle of 3 degrees, making it a 12-degree testing on assessment when considered from the fovea. A, 10-2 visual fields subtends a 10 degree angle and hence measures visual field indices in the 10 degrees. The average total macular thickness (in the 8×8 mm grid) of all subjects was 289(Table 13.7).

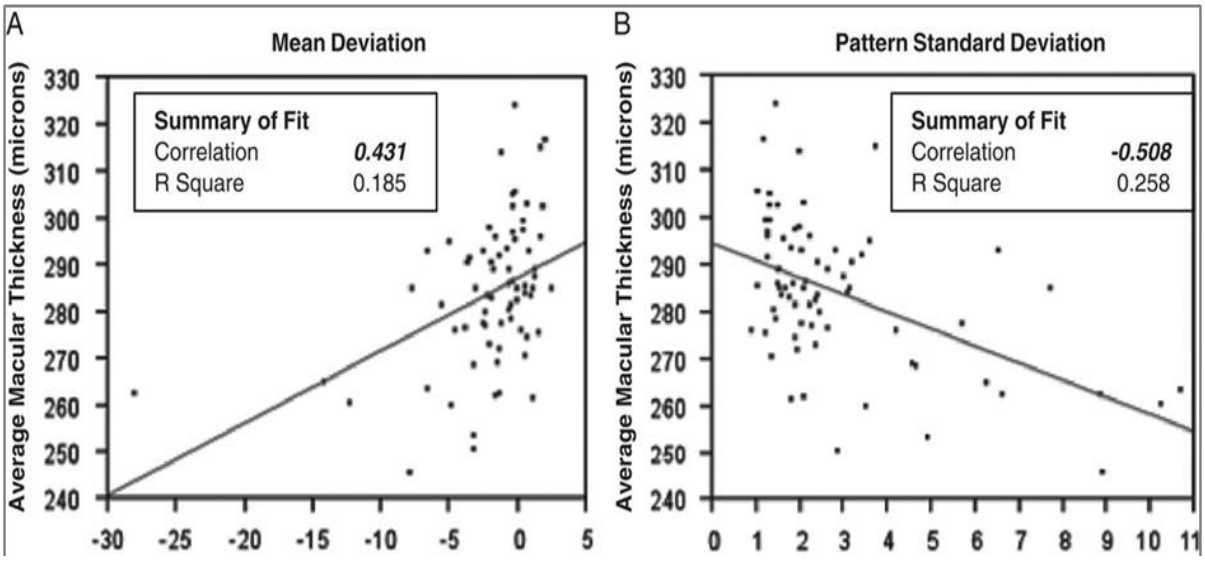
Eyes with thinner average total macular thickness (<270μm) had more negative MD scores (MD −7.76) and higher PSD scores (PSD 6.83), corresponding to significant visual field deficits. In eyes with average macular thickness >300μm, the visual field parameters were virtually normal (MD 0.89 and PSD 1.81). In eyes with intermediate thickness (between 270 and 300μm), the visual field parameters were also intermediate (MD −2.27 and PSD 3.39).

Table13.7: MD, mean deviation score on the Humphrey visual field; PSD, pattern standard deviation on the Humphrey visual field

N Define groups	Average macular thickness	Mean MD	Mean PSD
Thin group	<270 μm	−7.76 (SD 7.78)	6.83 (SD 3.04)
Intermediate group	270–300μm	−2.27 (SD 2.17)	3.39 (SD 1.32)
Thicker group	>300 μm	0.89 (SD 1.15)	1.81 (SD 0.83)

Statistical analysis was done on this data using a bivariate fit analysis.

Figure 13.8. Macular thickness correlates with mean deviation (MD) and pattern standard deviation (PSD) scores. A. Bivariate fit analysis of average total retinal thickness of the macula compared with the MD score of the Humphrey visual field for each study subject, shown by graphical plot. B. Bivariate fit analysis as in (A), showing the comparison of the macular thickness to the PSD score of the Humphrey visual field.



Bivariate fit analysis (Fig 13.8) shows a significant correlation between average macular thickness and both the MD score (Fig. 13.8A) and PSD score (Fig. 13.8B). Regarding the correlation between MD and macular thickness, the correlation coefficient is 0.431, R^2 0.185, linear fit (average macular thickness=287+1.55MD), with P = 0.0001. The correlation between PSD and macular thickness showed a correlation coefficient of −0.508, R^2 0.258, linear fit (average macular thickness=294–3.61PSD), with P< 0.0001.

Correlation of the asymmetry between the right and left visual fields with the asymmetry in total macular thickness between the eyes

Asymmetry in visual fields has not been explored in Multiple sclerosis until date. The difference in the average total macular thickness between the 2 eyes was calculated and compared with the difference of the global parameters of the HVF between the 2 eyes. The results show that patients with greater visual field defects in the right eye were found to have thinner macular thickness in that eye, and similarly, greater visual field defects in the left eye was associated with thinner macular thickness in the left (table 3). An absolute difference in MD score of 3 correlated with a difference in average macular thickness of approximately 11 to 13μm between the eyes.

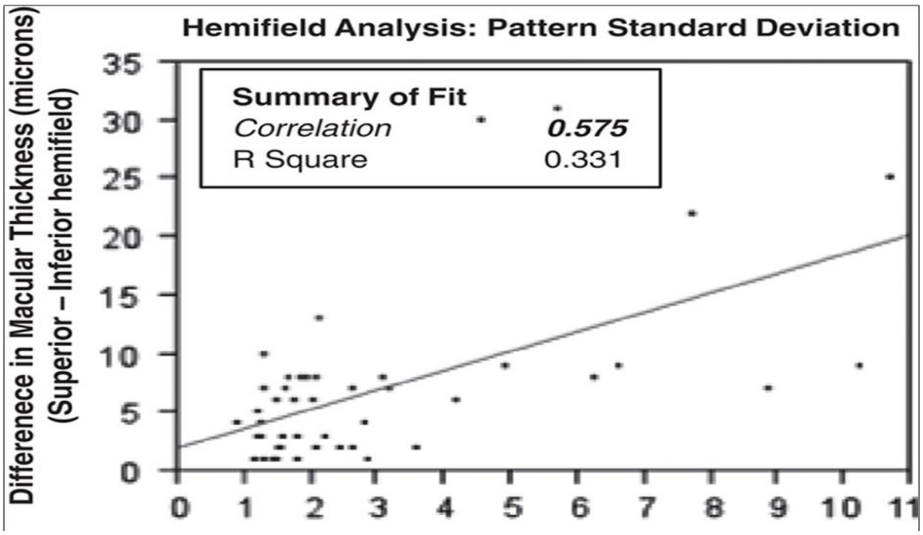
Table 13.9: Subgroup analysis of posterior pole OCT

Sub group analysis	Mean difference in MD score between eyes	Mean difference in average macular thickness (micron)	Mean macular thickness (micron) Right eye OD	Mean macular thickness (micron) Left eye OS
OD worse than OS (>3) N=24	9.28 (SD 8.97)	14 (SD 7)	266 (SD14)	276 (SD14)
OD similar to OS N=65	0.22(SD 1.26)	1(SD 7)	286 (SD15)	287(SD 16)
OS worse than OD(>3) N= 22	8.21(SD 4.33)	12(SD 12)	284 (SD16)	273 (SD23)

Correlation of the Asymmetry in the Macular Thickness of the Inferior Macula and Superior Macula with PSD Score

Asymmetry within each eye was observed by noting the difference in the average thickness of the superior macula and inferior macula (Fig. 3). The difference in macular thickness between the superior macula and inferior macula was compared with the PSD score of the visual fields. Greater asymmetry, or difference in thickness between the superior and inferior maculae, correlated strongly with a larger PSD score, both in the event of worse superior thinning and worse inferior thinning. For eyes with superior thinning, the correlation coefficient is 0.575, R^2 0.331, linear fit (difference in macular thickness=2.03+1.65PSD), with P< 0.0001 (Fig. 3), and for eyes with worse inferior thinning, the correlation coefficient is −0.697, R^2 0.367, linear fit (difference in macular thickness = −1.54–1.20PSD), with P = 0.0022.

Figure 13.10: Correlation fit between macular thickness and PSD



Asymmetry in macular thickness correlates with pattern standard deviation (PSD) scores. Bivariate fit analysis of the difference in average macular thickness (superior-half minus inferior-half) compared with PSD of the Humphrey visual field for each study subject, shown by graphical plot. This plot represents the data from subjects with superior macular thinning; results from subjects with inferior macular thinning are similar.

Correlation of the Asymmetry in Macular Thickness with Asymmetry in the RNFL

Given the current understanding of the pathophysiology of demyelinating optic neuropathy, correlation between RNFL defects and macular thickness is not yet fully known(Fig. 13.11, Table 13.11a). Subjects with a supero-temporal defect in the RNFL were more likely to have superior thinning of the macula (on asymmetry analysis comparing retinal thickness of the superior macula and inferior macula); the correlation coefficient is 0.746, R^2 0.557, linear fit (difference in sup-temp RNFL = 4.33 + 2.20×difference in macular thickness) with $P< 0.0001$ (Fig. 13.11a). Similarly, subjects with an infero-temporal RNFL defect were more likely to have inferior thinning of the macula on asymmetry analysis, with a correlation coefficient of 0.845, R^2 0.715, linear fit (difference in inf-temp RNFL = 4.21 + 2.36×difference in macular thickness), $P< 0.0001$ (Fig. 13.11b).

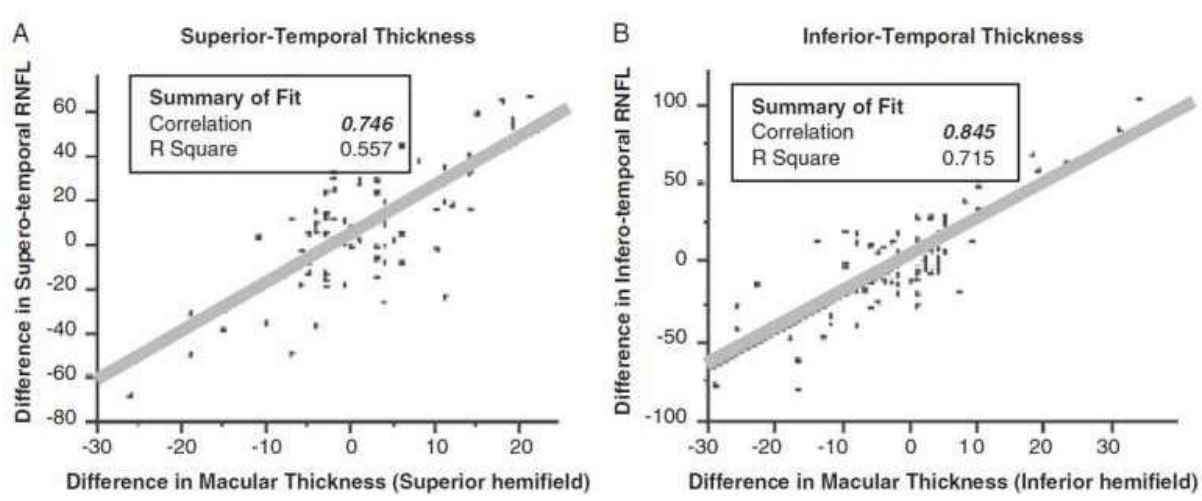


Figure 13.11 A and B

Retinal nerve fiber layer (RNFL) asymmetry correlates with asymmetry of macular thickness. A, Bivariate fit analysis of the difference in supero-temporal RNFL thickness between the right and left eyes compared with the difference in macular thickness of the superior-half between the 2 eyes, shown by graphical plot. B, Bivariate fit analysis as in (A), shows the comparison of the infero-temporal RNFL thickness with the thickness of the inferior-half of the macula.

13.4. Discussion

This study helps to further clarify the structure-function relationship between retinal thickness and visual field testing results. In this study, we have been able to demonstrate how the analysis, which is useful in identifying and quantifying areas of RNFL damage, correlates with visual function, specifically to HVF parameters.

Our data show that eyes with a total macular thickness of <270μm were significantly more likely to be associated with visual field loss, by both MD and PSD. However, eyes with a total macular thickness of >300μm were less likely to be associated with visual field losses. Asymmetry of the macular thickness, either when comparing the 2 eyes to each other or the superior macula to the inferior macula within the same eye, was significantly associated with a worse visual field localizing to the thinner retina or thinner retinal half, respectively. Finally, we were able to demonstrate the strong correlation between RNFL and macular thickness measurements.

Measurement of both the RNFL and the macular thickness provides additional useful information in the diagnosis and management of Multiple sclerosis patients. Further delineation of the structure-function relationship between retinal thickness and visual field testing could allow for a more objective diagnosis of MS by documenting loss of retinal tissue at the posterior pole, particularly in patients in an early stage of the disease or where a reliable visual field cannot be obtained. In addition, in cases where the diagnosis of MS is not yet made, a quantitative analysis of macular and RNFL thickness may provide key information that aids in the determination of the appropriate level of therapy required. The high intervisit reproducibility of the SD-OCT is useful in detecting small but significant changes during the course of observation or treatment. When interpreted in the context of an individual patient, the

additional information provided by the SD-OCT may alter clinical decision making in cases where the diagnosis or the extent of damage is not straightforward by other clinical parameters. This information can also be critical in guiding therapeutic decisions in patients who cannot do a reliable visual field examination.

In our study, the structure-function correlations between visual field parameters and macular thickness show how macular thickness as measured by SD-OCT can help in confirming the existence and extent of visual field defects. In this study, axial scans covering a 8 mm diameter area of the macula were acquired and the macula was divided into sectoral zones with a total of 64 zones protocol. We found a correlation between both the average macular volume and the average RNFL thickness with visual field MD. The data suggest that OCT of the macular is more reproducible than of the RNFL. Building upon these findings, our study shows a relationship between macular thickness, as measured by high resolution SD-OCT of a large area of macula (8 × 8 mm grid), and the MD and PSD of the HVF, well-established parameters for monitoring progression over time, offering a possible translation of structure into function. The ability to translate the structure of the macula into an understanding of its function is possible with SD-OCT. A method to compare structural and functional measures of damage using probability maps is another exciting advancement using OCT and visual field data.

As the ability to reliably measure these layers in patients improves, this will undoubtedly be the next step in refining our ability to correlate structure with function.

Asymmetry is important and has been established by this study in the diagnosis of Multiple sclerosis. Our protocol includes an 8 × 8 mm area of the macula. We have been able to show how within a single eye, asymmetry of retinal thickness (either thinner superior macula or inferior macula) correlates significantly with higher PSD. Asymmetry between the eyes also corresponds to visual field defects, with the eye with the thinner macula correlating to the higher MD. Demonstration of asymmetry in an objective test such as the OCT may help the patient's understanding of the severity of disease, perhaps ultimately improving the approach to treatment by clinicians.

Finally, the analysis of the visual fields relied on parameters of MD and PSD, which are global measures. The PSD has a parabolic distribution and given the large spectrum of the disease represented in this group, our correlation analyses may underestimate the association between this visual field parameter and the retinal thickness. An analysis using individual numerical data points of the HVF provides stronger structure-function correlation of macular thickness to visual field deficit.

In conclusion, our results show a significant point-to-point correlation between the visual field sensitivity and RNFL. Further study using the Short wavelength automated perimetry will help in further precision in certain subtypes and determining response to disease modifying treatment.

Chapter 14

Final Results: Structure meets Function

“Eye is a window to the brain”

Vision in Multiple Sclerosis (MS):

14.1. Structure-Function Correlations

Visual dysfunction is one of the most common clinical manifestations of multiple sclerosis (MS). We assessed structure and function of visual system and correlated it with function of the neurological system

Function of visual system was assessed using:

1) The LogMAR chart
2) Sloan 2.5% and Sloan 1.25% chart
3) The HRR color vision chart and
4) Humphrey's visual field analyser.

The structure of the Visual system was assessed using:

1) The ocular coherence tomogram of the macula, to assess the RNFL in the macula, macular volume and thickness of various layers of retina using the auto-segmentation function on OCT.
2) The EDI protocol of OCT was used to determine the choroid thickness in each eye.
3) The OCT, Optic nerve head protocol was used to scan the optic nerve and peri-papillary area for RNFL assessment.
4) Posterior pole OCT.

The neurological function was assessed using:

1) EDDS
2) QOL short questionnaire

14.2 Statistical Methods:

The individual chapters explored the findings in case and control in a structured fashion, exploring and presenting on;

1) Descriptive statistics
2) Checking the spread of data and its normality
3) Applying bivariate analysis for qualitative data
4) Describing the ROC curve when appropriate
5) Applying correlation to the group either Pearson or spearman as appropriate.

In this final chapter, we try to explore the effect of multiple factors relating to structure and function of visual pathway and neurological function. SPSS has been used and a multivariate regression model with 2 tailed significance of p less than 0.05, taking into consideration the adjusted p values on Bonferroni's correction.

Odds ratio has been depicted to determine the predictive value of each of the test in segregating the control population from the diseased.

We have applied the broken stick model for relation between the RNFL and VF test, as this has not yet been explored in MS. The tipping points have been determined and scatter plots have been used to explore the points at which the loss of retinal nerve fiber layer becomes significant as evident by worsening of function.

One eye or two eye model

Studies pertaining to eyes usual generate 2-fold data in most cases due to the binocular nature. There has always been debate surrounding one eye data or two eyes. We have considered one eye or two eye model based on the outcome being correlated. For example, binocular data has been considered for comparison with EDSS and quality of life, while uniocular data has been used for most visual parameters. The overall effect of vision has then been explored by binocular data. Inter eye correlations has been used when appropriate.

14.3. Results:

Composite study of structure with function

Multiple sclerosis is a complex disease with many unanswered questions in relation to diagnosis, prognosis and response to treatment. Studies in MS have tried to answer the questions by studying genetics, environmental factors, neurological parameters such as EDSS, MSFC and cognitive testing, psychophysical tests and recent numerous studies on Magnetic Resonance Imaging.

There is no single study in the UK that has looked at the visual pathway (part of the forebrain) in such detailed fashion, not only assessing structure and function of the vision in finer details, but also correlating it with important parameters of neurological function. The study over-all makes suggestion on visual parameters to be considered for early diagnosis, predicting prognosis and also to evaluate response to disease modifying treatment.

The initial part of this section sums up the overall interaction of functional and structural visual parameters with the later part correlating it with key neurological tests.

We suggest visual improvement and loss by the low-contrast acuity chart if there has been a 7-10 letter change in the overall visual scores. The importance of Sloan 1.25% chart and Sloan 2.5% chart in detecting visual changes cannot be emphasised enough. Sloan forms a sensitive test for asymptomatic visual loss in MS.

Assessment of colour vision using the HRR chart helps to specify the type of colour deficiency and also drives home the concept of asymptomatic loss of colour vision. The HRR chart aims to target detection of deficit along the Magnocellular, Parvocellular pathway, paying special attention to blue yellow, Koniocellular pathway.

The Mean deviation on Humphrey's visual field shows correlation with the Sloan and HRR colour testing. The Blue yellow pathway which is most likely to be affected in MS can be tested with Sloan 1.25% chart along with HRR colour chart and the SWAP (short wavelength automated perimeter) testing on visual field which specifically tests the target blue yellow pathway.

The RNFL loss as has shown by OCT parameters correlate very well with outcomes on Sloan chart, HRR colour chart and visual field indices. The study also generates the importance of posterior pole testing protocol of OCT in symptomatic and asymptomatic optic neuropathy of multiple sclerosis.

EDI-OCT has never been explored in MS and this study addresses the most important vascular tissue of the eye, the choroid. We have interesting preliminary findings on the choroid thickness in controls and disease and hoping this will open the door to a similar study with bigger numbers to establish the connection proposed in this study. Is choroid the hub for starts of immunological process and break down of blood retinal barrier!

The RNFL axonal loss in patients with MS and controls are shown in Table 14.1, depicting the magnitude of differences in means for OCT values.

Table 14.1: Depicting the magnitude of differences in means for vision and OCT values.

Parameter	Control Mean	Disease Mean	ON positive	DMT group
LogMAR	57	42	42	43
Sloan 2.5%	49	22	12	21
Sloan 1.25%	43	13	5	8
MD	-2.13	-4.74	-6.75	-5.2
PSD	-1.13	-5.4	-7.89	-5.2
M.Vol	9.2	7.9	6.43	8.1
RNFL	29.6	23.99	21.65	25.3
GCIP	88.9	82.43	79.35	85.2
EDI	261	262	185	254
PPRNFL	92.76	85.91	79.75	82.2

MD=Mean deviation, PSD=pattern std deviation, M.vol=macular volume, RNFL= retinal nerve fibre layer, GCIP= ganglion cell inner plexiform complex, EDI= Enhanced depth imaging and PPRNFL= peripapillary RNFL.

The logistic regression on this composite data and the subsequent odds ratio calculation after application of Bonferroni's correction is depicted in figure 14.2.

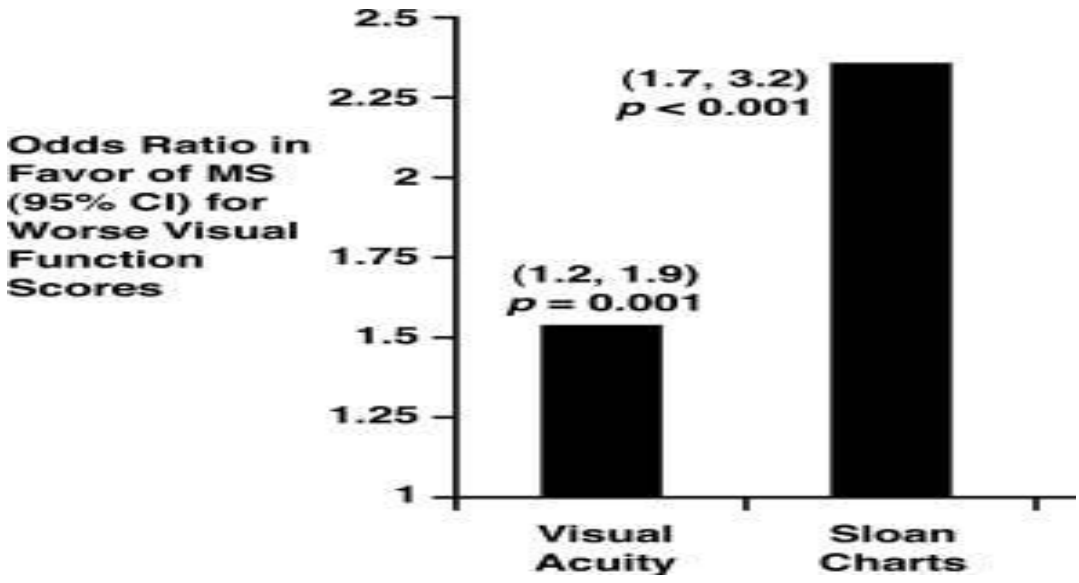


Figure 14.2

Logistic regression analyses demonstrating capacity for Sloan visual function test to predict multiple sclerosis (MS) vs disease-free control status in a heterogeneous MS cohort, accounting simultaneously for age. Odds ratios in favor of participants with worse vision scores being MS patients (versus disease-free controls) were greatest for low-contrast letter acuity (Sloan charts). It distinguishes MS patients from disease-free control subjects, even after accounting for age differences between the two groups.

This study provides the data demonstrating that low-contrast acuity scores capture unique aspects of dysfunction not captured by standard neurologic scales of EDSS or the MSFC, suggesting that adding a visual component to the MSFC would increase the applicability of this measure.

Vision-Specific Quality of Life (QOL) Measures

The role of patient related measures on quality of life and vision has not been explored completely. Irrespective of the disease, it is very important to get the patient perspective in disease and how it affects their quality of life and vision. As this study is already looking into multifactorial aspects, we designed our own quality of life and vision assessment questionnaire as shown below. This was further related to structure and function parameters of vision and MS. Collectively, these data demonstrated that low-contrast acuity testing provides information on patient-reported aspects of vision. This is a key feature for clinical measures and a prerequisite for their use as primary outcomes in clinical trials.

Table 14.5: Quality of Life / Vision and its correlation to EDSS and Sloan's chart

Cases N=55	Unaffected	Affected , but managing	Affected, not managing
Quality of vision	15	32	8
Quality of life	2	37	16
Binocular Sloan chart 1.25%	12 (VA<6/12)	36 (VA 6/12-6/36)	4 (>6/36)
EDSS	4(EDSS <3)	46(EDSS 3-6)	5(EDSS >6)

Quality of life and binocular vision outcome are highly corelated , making assessment in visual outcome more depictive of the patient outcome measures.Quality of vision on the other hand is a highly sensitive measure which affects many patients even before it is evident on the Sloan binocular chart. EDDS is a crude measure of patient outcome and shows a wide variation to quality of life and outcome measures.Importantly, greater degrees of binocular summation were predictive of better patient outcome by the QOL (P=0.02) and QOV (P=0.03), indicating that the capacity to use both eyes together is an important factor in determining how well patients with MS can perform daily activities.

This suggests assessing patients quality of life and vision on an yearly basis using the standard MS-QOL 54 and NEI-VFQ-25 is crucial and informative. Patient outcome measures have not been used to its best potential until date.

Larger studies and additional meta-analyses will allow us to further refine the precision of these representative average values.

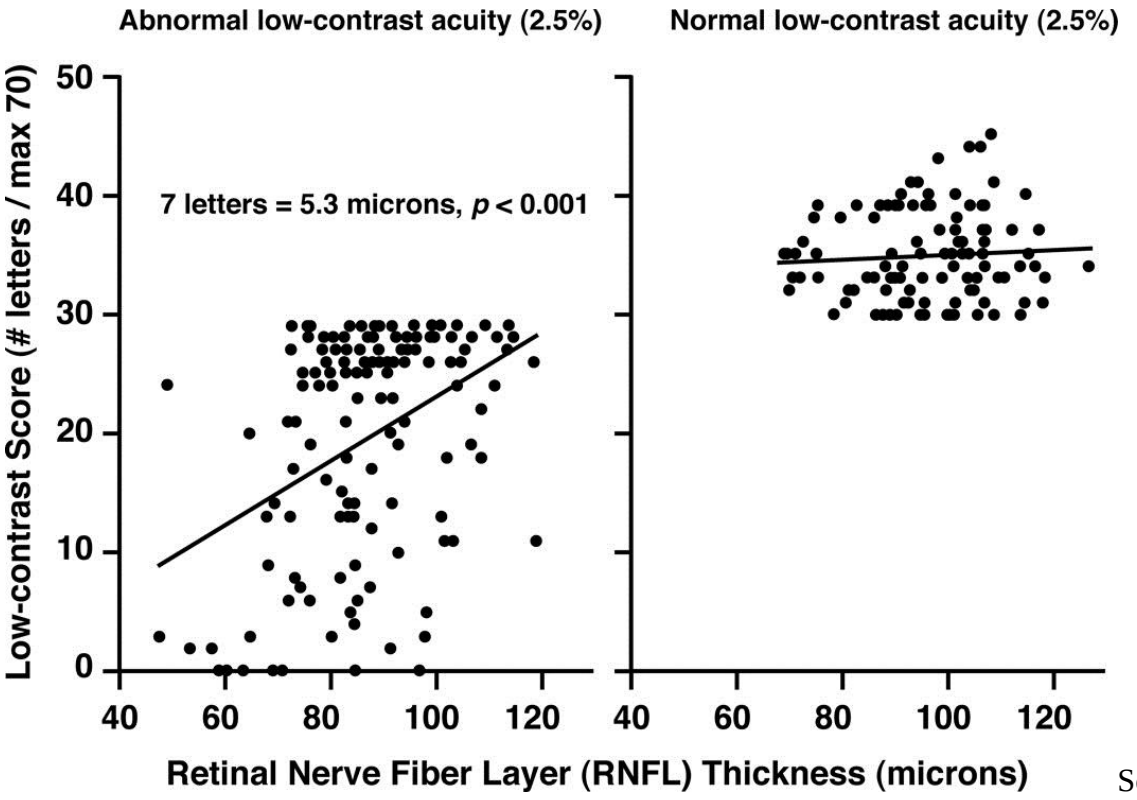
OCT Investigations in MS and ON

In our study, which utilized spectral domain OCT to assess the RNFL. 45 eyes with MS who had a previous event of acute optic neuritis (ON) were analysed. The thickness of the RNFL was shown to be reduced by 46% in the affected eyes of the patients with MS versus the control eyes (P<0.01), and by check 11% when affected eyes were compared with the 'unaffected' eyes of the same patient (P<0.01). Even in the clinically unaffected eyes of patients, however, there was a 26% reduction in RNFL thickness when compared with control eyes (P<0.01). check whole para

This finding is striking given that the RNFL is only about 110–120 μm thick by the age of 15 years, and that most healthy individuals (without a history of glaucoma or macular degeneration) will lose only about 0.017% per year in retinal thickness, which equates to approximately 10–20 μm over 60 years

Testing patients with Low-contrast letter acuity in MS eyes with a history of acute ON, we found a threshold for abnormal visual function and axonal loss at 85μm (Fig. 14.7).

Figure 14.7: Threshold for abnormal visual function and axonal loss



Scatter plots showing relation of low-contrast letter acuity to retinal nerve fiber layer (RNFL) thickness in microns for eyes from a heterogeneous MS cohort with a history of acute optic neuritis (ON). The left panel shows eyes with abnormal low-contrast at 2.5% contrast. Among eyes with abnormal low-contrast acuity, worse scores were significantly associated with lower (thinner) peripapillary RNFL, accounting for age and adjusting for within-patient, inter-eye correlations.

The time course of RNFL axonal loss following an episode of acute ON is an important for determining the "window of opportunity" within which a neuroprotective agent should be administered in a treatment trial.)

One of the most important findings that has resulted from the use of OCT MS studies is the correlation between RNFL thinning and visual loss, as measured

by low-contrast letter acuity. We found that RNFL thickness was reduced significantly among MS patients (92 µm) versus controls (105 µm, P<0.001), and particularly reduced in MS ON eyes (85 µm, P<0.001)

Assessment done on the Optic nerve head OCT revealed interesting results. Sectoral RNFL atrophy was a striking feature in our cohort of patients. There was an evidence of significant drop in the inferior and superior nasal fibres and widening of vertical cup disc ratio. The optic nerve looked overall pale with inferior and superior rim thinning resembling the disc of glaucomatous optic neuropathy. This possible reflects that the final pathway for the death of neurons in MS may be similar to that in glaucoma .For those interested in optic neuropathies, the comparison of optic neuropathy in Glaucoma to that of Multiple sclerosis may further be explored using the Glaucoma premium model OCT protocol on Spectralis OCT.

Table14.8: Comparison of sectoral RNFL in disease and control:

Mean values microns	Superior RNFL	Inferior RNFL	Nasal RNFL	Temporal RNFL
Control N=50	98.8	102	63	54
Disease N=110	83	78	59	49

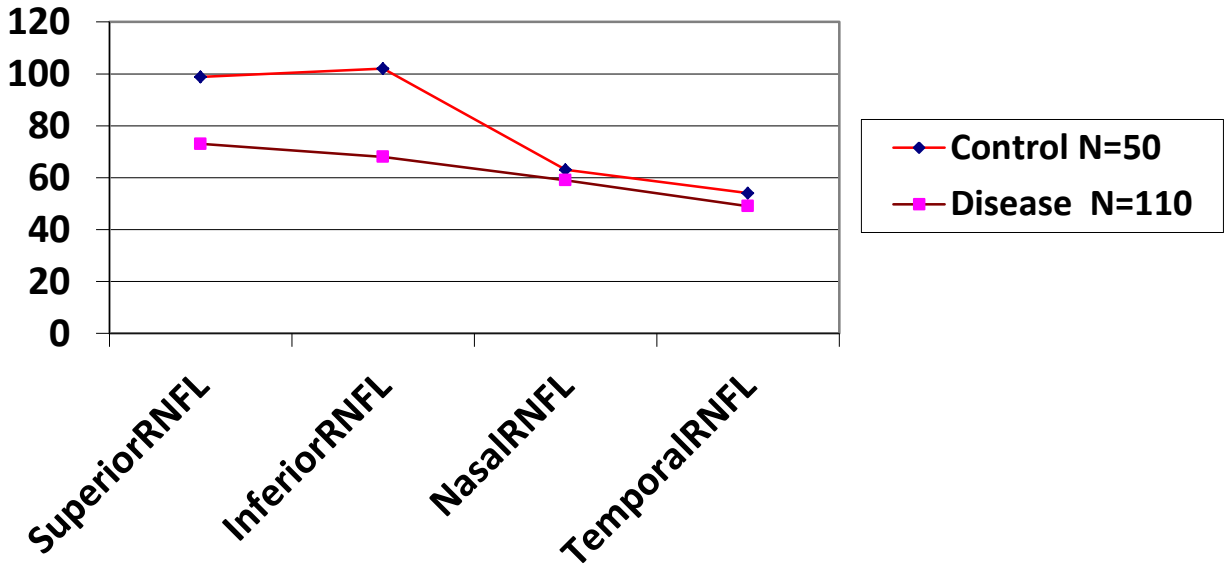
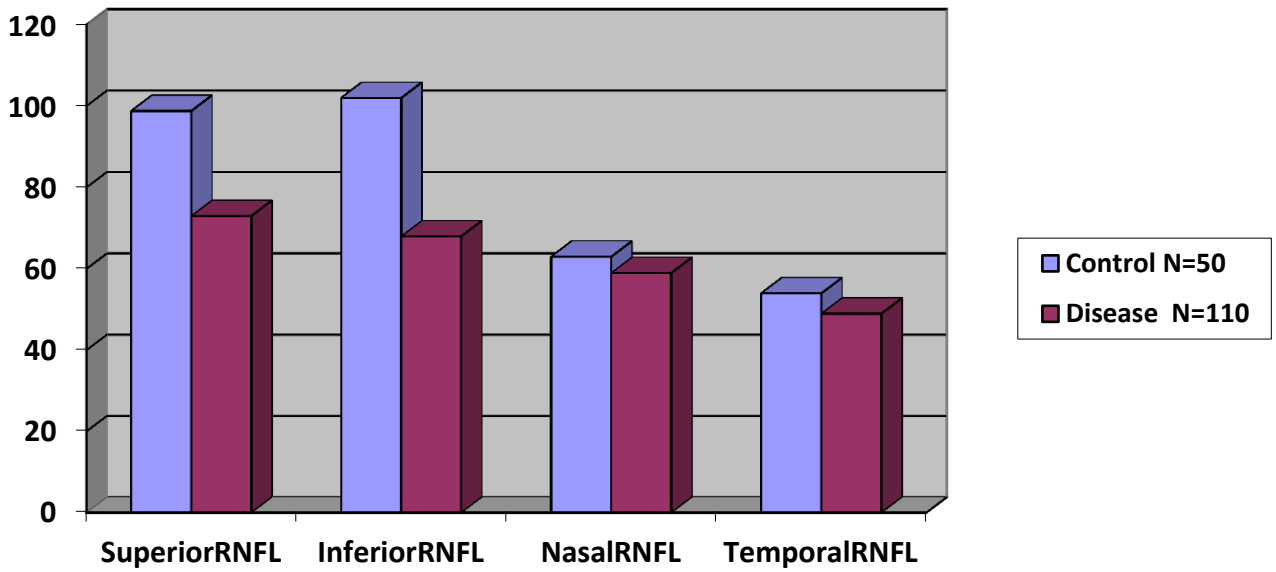


Figure 14.9: Predominant loss in the superior and inferior rim, inferior more marked in this cohort.



14.10: Bar chart depicting the intensity of loss of superior and inferior nerve fiber in disease

Chapter 15: The Final conclusion

Final conclusion:

Multiple sclerosis is a progressive disease leading to significant disability affecting quality of life and has a huge social and financial burden on the department of health.

Vision studies in our project has shown to be very specific and sensitive in detecting disease and to determine the prognosis in RRMS .Visual assessments have a synergistic value to other tests such as MSFC, EDSS and MRI.

Visual charts

Snellen's chart has been a very routinely used chart in most NHS trust across England and Wales. The UHNM has now moved on to using the ETDRS charts since 2013.The ETDRS chart is a LogMAR chart that has been used in the research project to compare it with the Sloan 2.5% and 1.25% contrast charts. Sloan chart are not a routine visual testing method and is in use only at the level of clinical trials in Multiple sclerosis.

Snellen's chart has shown to have a poor specificity and sensitivity in detecting the control and the disease. The limitations of the test chart take an upper hand and make it a less reliable method of vision testing.

LogMAR chart is comparable to the Sloan 2.5% chart, though the 1.25% chart has showed to more sensitive with a high odds ratio .The ROC curve also shows the rate of identification of disease versus control to be better on Sloan 1.25% chart. Sloan 1.25% has the ability to identify visual defects at an early stage and hence this chart may see a good use in the visual pathway assessment in future.

We are still using the Ishihara charts to detect colour deficit in all eye conditions. Ishihara along with Snellen's make a very poor combination of visual tests giving very little information of the condition. Why we still continue to use it, is not very clear. As we all know, changing old practise is hard and takes time. Hope this is the era for the change in approach to visual assessment in MS.

The HRR chart showed color deficits of varying nature in the positive and negative optic neuritis cohort, with an ability to detect the type and extent of colour deficit. The HRR Chart has a potential of detecting early changes in the very sensitive koniocellular pathway even before it is evident on visual testing by Sloan 2.5% chart. The HRR chart and Sloan 1.25% chart form a good pair of

test to determine the prognosis of disease and in detection of changes in asymptomatic patients. Studies with bigger numbers are required to explore the potential of the 2 tests in combination.

Age at optic neuritis seem to have an effect on the outcome with early onset optic neuritis showing better prognosis than the late onset type.

We would like to explore this further in our future studies.

OCT is the future in assessment of visual pathway in MS. It is sensitive with high inter and intra test reliability.

The RNFL layer and macular volume seem to be sensitive in detecting changes in the un myelinated RNFL. Much has been spoken about the GCIP and manual/ automated segmentation, though, our research showed not much gain from it. Additionally, it is time consuming and can make OCT bit complicated to those who are trying to incorporate OCT in their day to day protocol.

The posterior pole OCT has not been spoken of in previous research work globally. Our research project found a great potential for the Posterior pole protocol OCT and the element of structure comes to life when compared with function as tested by the visual field sensitivity on Humphrey's field test. Work needs to be done, though we had significant p value correlating both the parameters. The down side of this combination is the time consumption of visual field and the fatigue that can be exaggerated for patients with MS. In our

cohort, we had reliable field at the first attempt though, we think, it may be variable if the cohort of patients included SPMS and PPMS.

EDI OCT has been used in our study as MS is an autoimmune demyelination. A contribution from the choroid was thought as it is embryologically similar to the choroidal plexuses in the brain.

Our study is the first of the kind which shows the thickness of the choroid to be variable. We are not able to strongly prove that EDI OCT is very diagnostic due to the low recruitment score and also that we did not give much importance at the time of conducting the research examination.

More studies from our group will be done in future on EDI OCT depending on staffing, ethics and interest of future researchers.

We have specific interest in studying the so called OSMS group. We plan to study the Aquaporin genes along with MRI and EDI OCT in the future vision studies.

This study was designed in 2009 even before any study from the American group was published on structure and function. Funding and ethics are a major hindrance to the take-off of a research project in the UK. Our study also

underlines the importance of patient related measure of quality of life and vision which adds more value than any testing parameters.

It is very important to know what this parameter on vision translates in the day to day life of a patient, without which most tests are incomplete.

15.4. Revisiting the key points.

1. Structural loss of neurons in the anterior visual pathway as determined by OCT correlates with the functional loss of vision as determined by Sloan's contrast chart.
2. HRR (Hardy Rand Rittler) colour chart is a very sensitive test for colour vision in Multiple sclerosis and Optic neuritis. It not only detects symptomatic and asymptomatic optic neuritis, but also, grades the extent of damage.
3. Age of onset of optic neuritis in MS determines the rate at which ganglion cell layer is lost. The results of patients with early onset optic neuritis have better outcome measure compared to the late onset type of optic neuritis. This may relate to the variation in macrophagic activity in the optic nerve in the younger age group.
4. The thickness of choroid in MS patients, as measured by the EDI OCT, is significantly variable than the control groups. A further study with a bigger sample size is indicated by this project.
5. Posterior pole thickness on OCT closely correlates to the sensitivity (point-point) of the visual fields as tested by SITA standard Humphrey's visual fields testing. This positive correlation can be exploited in early diagnosis and response to treatment in MS.

6. RNFL thickness as determined by the serial OCT's of the optic nerve head is a valuable tool to diagnose asymptomatic optic neuropathy.
7. Alterations in the ganglion cell layer thickness and inner nuclear layer thickness is directly related to functional measures in MS.
8. Retinal segmentation OCT findings do not give any extra information when compared to other functional scores on visual testing.

Publications:

1) Optico-spinal MS in British Caucasians (First Author): A study of clinical features, radiological features and genetics relating to NMO antibodies. Multiple Sclerosis Journal, October 2011; vol. 17, 10 suppl: pp. S507-S524.
2) Progression of disability in multiple sclerosis: A study of factors influencing median time to reach an EDSS value. Multiple Sclerosis and Related Disorders, Volume 2, Issue 2, Pages 109-116 , Oct 2012.
3) Increased frequency of IL17-IL22 dual secreting Tcells in relapsing/remitting multiple sclerosis. (2014)
   doi: 10.1177/1352458514547848
   Mult Scler September 2014 vol. 20 no. 1 suppl 501-510

International Posters and Presentations

4) International presentation (first author) and poster at the European and American society for research in MS (2011) in the highly privileged late breaking news section of the meeting at Gothenburg, Sweden. "Optico-spinal MS in British Caucasians: A study of clinical features, radiological features and genetics relating to NMO antibodies".
5) "NMO antibodies in Optico spinal MS": Presentation (first author) at 2012 European Charcot's meeting for Young investigators award.

6) ACTRIMS-ECTRIMS Boston 2014: Late Breaking News
   Increased frequency of IL17-IL22 dual secreting Tcells in relapsing/remitting multiple

Grant applications as primary author

7) Primary author for a grant application through SHINE (UK). We aimed to unravel an exciting phenomenon of CCSVI- chronic cerebrospinal venous insufficiency in MS.
8) Submitted a fellowship grant application through the ECTRIMS committee 2012.

Nominations

9) I have been nominated for the European young investigators award and the West Midlands Vitae prize.

Sub Investigator to Keele MS Clinical Trial Unit:

I have been a sub-investigator to 13 clinical trials as an Ophthalmologist with Neurostatus qualification for clinical trials.

1) Phase II study of oral Fingolimod (FTY720) in multiple sclerosis and a 24-month extension of a phase II trial assessing the efficacy, safety, and tolerability of the once-daily oral sphingosine-1-phosphate receptor modulator, Fingolimod, in relapsing multiple sclerosis (MS). I also contributed to monitoring the macular oedema on OCT**.**
2) A PIC site for a Phase II Double Blind, Randomized, Placebo Controlled Trial of Neuroprotection With Phenytoin in Acute Optic Neuritis.
3) A randomized, double-blind, double-dummy, parallel-group study to evaluate the efficacy and safety of Ocrelizumab in comparison with Rebif (interferon beta-1a) in patients with relapsing multiple sclerosis. (OPERA)
4) A Phase III, randomized, double-blind, placebo-controlled, multi-centre clinical trial of oral Cladribine in subjects with a first clinical event at high risk of converting to MS (ORACLE MS).
5) A Double-Blind, Multicenter, Extension Study to Evaluate the Safety and Efficacy of DAC HYP in Subjects with Multiple Sclerosis Who Have Completed Treatment in Study 205MS201 (SELECT-re study)
6) An open label multicenter, extension study to evaluate the safety and tolerability of Natalizumab following re-initiation of dosing in multiple

sclerosis subjects who have completed study C-1801 or C1802 and a dosing suspension safety evaluation.(Tysabrii)

7) Long-term extension of the multinational, double-blind, placebo controlled study EFC6-40 (HMR 1726D/3001) to document the safety of two doses of Teriflunomide (7 and 14mg) in patients with multiple sclerosis with relapses (TEMSO ext. study)
8) Long-term extension of the multinational, double-blind, placebo controlled studies PDY6045 and PDY6046 to document the safety of Teriflunomide when added to treatment with interferon or Glatiramer acetate in patients with multiple sclerosis with relapses
9) A randomised double blind placebo-controlled parallel group multi-centre trial of Cannabinoids to slow progression in multiple sclerosis. (CUPID study).
10) Multinational, Multicentre, Randomised, Double-Blind, Placebo Controlled, Parallel Group Study to evaluate the effect of early Glatiramer acetate treatment in delaying the conversion to clinically definite multiple sclerosis of subjects presenting with a clinically isolated syndrome (PRECISE study)
11) A Phase III, Double-Blind, Placebo-Controlled, Multicentre, Parallel Group, Extension Trial to Evaluate the Safety and Tolerability of Oral Cladribine in Subjects with Relapsing-Remitting Multiple Sclerosis Who Have Completed Trial 25643 (CLARITY)

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Name	EDSS	ON		logMAR	2.50%	1.25%	COLOR
LS BE		Y SEVERE		50	33	22	
LS RE				45	33	22	NORMAL
LS LE				30	11	0	NORMAL
KS BE				60	13	24	
KS RE		YES		50	18	24	NORMAL
KS LE		NO		55	45	46	NORMAL
JB BE		YES		55	34	20	
JB RE		Y		55	25	0	MILD RG
JB LE		Y		55	30	14	NORMAL
CB BE				51	45	45	NORMAL
CB RE		AMBLYOPIA		30	20	25	NORMAL
CB LE		YES	1988	50	30	45	NORMAL
SCR BE				57	60	9	
SCR RE		YES, SEVERE	2004	30	0	0	MEDIUM RG
SCR LE				57	22	9	
CS BE		N		55	6	0	NORMAL
CS RE		N		50	6	0	NORMAL
CS LE		N		55	14	0	NORMAL
MA BE		N		41	34	25	NORMAL
MA RE		N		41	30	0	NORMAL
MA LE		N		34	28	25	NORMAL
SB BE				60	30	25	NORMAL
SB RE				60	30	25	NORMAL
SB LE				45	20	15	MILD RG DEUTAN
KM BE				60	40	40	NORMAL
KM RE				60	40	30	NORMAL
KM LE				55	30	25	NORMAL
PD BE				40	3	0	RG/BY STRONG
PD RE				30	0	0	RG/BY STRONG
PD LE		Y	1994	25	0	0	RG/BY STRONG
JA BE		N		51	25	15	NORMAL
JA RE				49	25	15	NORMAL
JA LE				32	10	11	MILD DEUTAN
JT BE		Y	2004	45	16	6	
JT RE				45	14	1	MILD RG
JT LE				42	1	2	MILD RG
AC BE		N		60	43	35	NORMAL
AC RE				55	29	30	NORMAL
ACLE				50	25	25	NORMAL
PE BE		N		60	45	30	NORMAL
PE RE				60	39	24	NORMAL
PE LE				55	38	23	NORMAL

SS BE	N		60	35	25	NORMAL
SS RE			55	30	9	NORMAL
SS LE			55	30	4	NORMAL
KT BE	N		50	24	15	NORMAL
KT RE			45	20	5	NORMAL
KT LE			40	10	1	NORMAL
DG BE	Y	2000	51	35	35	
DG RE			44	25	4	MILD RG
DG LE			48	30	10	NORMAL
VD BE			50	40	30	
VD RE	Y	2012	50	24	20	MILD RG
VD LE	Y		50	25	10	MILD RG
JC BE	N		55	40	20	NORMAL
JC RE			55	30	2	NORMAL
JC LE			50	25	2	NORMAL
NW BE	N		55	41	35	NORMAL
NW RE			50	39	20	NORMAL
NW LE			50	41	31	NORMAL
BS BE			50	40	40	NORMAL
BS RE			50	40	40	NORMAL
BS LE			50	40	40	NORMAL
DN BE			50	30	30	NORMAL
DN RE			40	14	9	NORMAL
DN LE	AMBLYOPIA		20	0	0	NORMAL
X1 BE	Y	1994	55	35	30	NORMAL
X1 RE			50	30	20	NORMAL
X1 LE			54	34	25	NORMAL
XE BE			50	25	10	
X2 RE	Y	2010	45	15	0	MILD RG
X2 LE			50	25	10	NORMAL
X3 BE	N		45	25	10	NORMAL
X3 RE			32	20	10	NORMAL
X3 LE			45	20	10	NORMAL
X4 BE	Y	2012	35	20	8	MILD RG BE
X4 RE			25	15	6	
X4 LE			32	18	5	
X5 BE	N		35	40	15	NORMAL
X5 RE			25	35	15	
X5 LE			32	40	10	
X6 BE	Y	1990	50	35	25	MILD RG
X6 RE			54	30	15	
X6 LE			45	29	12	
X7 BE	N		43	35	25	NORMAL
X7 RE			42	35	24	
X7 LE			43	32	25	
X8 BE			60	25	9	

X8 RE			58	24	10	
X8 LE	Y	2007	23	3	0	MOD RG
X9 BE	N		43	13	8	NORMAL
X9 RE			35	13	8	
X9 LE			42	12	8	
X10 BE	N		50	35	10	NORMAL
X10 RE			45	32	10	
X10 LE			43	35	8	
X11 BE			45	25	10	
X11 RE	Y	1990	15	3	1	MILD RG
X11 LE			43	22	10	
X12 BE	N		22	1	0	MILD RGR
X12 RE			12	1	0	
X12 LE			21	1	0	
X13 BE	Y	1994	35	6	1	MOD RG
X13 RE			35	3	0	
X13 LE			35	6	1	
X14 BE	N		56	34	24	NORMAL
X14 RE			55	35	21	
X14 LE			55	35	21	
X15 BE	N		37	15	12	NORMAL
X15 RE			36	13	13	
X15 LE			36	15	12	
X16 BE	Y	1996	5	0	0	N/A
X16 RE			3	0	0	
X16 LE			4	0	0	
X17 BE	N		60	45	35	NORMAL
X17 RE			58	43	35	
X17 LE			59	43	36	
X18 BE	N		45	22	16	N
X18 RE			32	21	16	
X18 LE			43	22	14	
X19 BE	Y	2012	3	0	0	NORMAL
X19 RE			4	0	0	
X19 LE			2	0	0	
X20 BE	N		60	45	35	NORMAL
X20 RE			58	46	36	
X20 LE			54	43	35	
X21 BE	Y	2013	54	10	3	MILD RG
X21 RE			55	8	1	
X21 LE			53	4	1	
X22 BE			54	25	4	
X22 RE	Y	2009	23	5	0	MILD RG
X22 LE			52	20	3	
X23 BE	N		45	26	18	NONE
X23 RE			32	20	17	

X23 LE			32	22	18	
X24 BE	N		22	3	0	NONE
X24 RE			12	0	0	
X24 LE			22	3	0	
X25 BE			54	45	35	
X25 RE			53	43	35	
X25 LE	Y	1990	54	45	35	
X26 BE	N		21	3	2	NORMAL
X26 RE			12	3	1	
X26 LE			22	1	1	
X27 BE	Y	2003	21	4	1	NORMAL
X27 RE			17	2	1	
X27 LE			14	2	0	
X28 BE	N		54	11	5	NORMAL
X28 RE			53	11	5	
X28 LE			51	11	5	
X29 BE			43	3	1	MOD RG
X29 RE	Y	2006	12	0	0	
X29 LE			42	2	1	
MEAN			42.55 mean	22.56954	14.07947	
MEDIAN			45	25	10	
SDP			14.67516	14.53541	12.87974	
IQR-			19.5	25	23.5	

Name	m-vol	edi	Mrnfl	gcip	pprnfl	ON	1.25
LS BE						Y SEVERE	
LS RE	8.84	215	22.6	78.8	92		
LS LE	8.76	235	21.4	76.2	91		
KS BE							
KS RE	7.23	280	18.3	72.2	60	YES	
KS LE	7.46	295	23.2	84.2	82	NO	
JB BE						YES	
JB RE	8.64	265	25.4	85.4	91	Y	
JB LE	8.77	220	26.8	86.3	96	Y	
CB BE							
CB RE	8.59	270	26.6	89.9	110	AMBLYOPIA	
CB LE	8.44	290	21	74.2	93	YES	1988
SCR BE							
SCR RE	6.88	342	11.1	64	60	YES, SEVERE	2004
SCR LE	7.65	265	27.9	89	77		
CS BE						N	
CS RE	8.3	272	28.4	91.2	86	N	
CS LE	8.54	243	26.8	87	87	N	
MA BE						N	
MA RE	9.47	159	32.3	94	85	N	
MA LE	6.88	356	22.2	81	73	N	
SB BE							
SB RE	7.5	260	24.6	88	82		
SB LE	7.1	292	21.7	84	78		
KM BE							
KM RE	7.71	221	26.2	94	98		
KM LE	7.65	265	25.8	94	94		
PD BE							
PD RE	10.17	117	29.2	104	63		
PD LE	8.51	283	20.01	79	64	Y	1994
JA BE						N	
JA RE	7.87	280	19.34	74	75		
JA LE	7.87	320	18.64	73	69		
JT BE						Y	2004
JT RE	7.73	310	16.7	69	63		
JT LE	7.78	298	18.56	78	68		
AC BE						N	
AC RE	8.68	220	28.7	89	104		
ACLE	8.76	234	30.23	91	102		
PE BE						N	
PE RE	8.7	242	30.98	92	70		
PE LE	8.77	247	31.07	93	94		

SS BE							N	
SS RE	8.88	210	29.5	96	103			
SS LE	9.03	140	32.54	97	102			
KT BE							N	
KT RE	8.34	284	25.6	86	103			
KT LE	8.54	275	26.4	89	96			
DG BE							Y	2000
DG RE	7.95	339	19.3	76	60			
DG LE	8.78	250	26.3	95	78			
VD BE								
VD RE	8.5	236	20.6	77	110		Y	2012
VD LE	8.56	223	23.7	85	111		Y	
JC BE							N	
JC RE	9.1	263	29.6	96	96			
JC LE	9.26	263	31.9	98	100			
NW BE							N	
NW RE	8.7	220	32.9	97	120			
NW LE	8.2	280	28.9	93	96			
BS BE								
BS RE	9.8	160	28.6	87	103			
BS LE	9.7	175	29.7	89	101			
DN BE								
DN RE	8.43	252	28.7	79	85			
DN LE	8.59	213	29.7	81	83		AMBLYOPIA	
X1 BE							Y	1994
X1 RE	7.65	297	18.6	72	65			
X1 LE	7.86	265	22.3	83	76			
XE BE								
X2 RE	6.8	318	16.1	71	58		Y	2010
X2 LE	7.65	265	21.7	81	78			
X3 BE							N	
X3 RE	8.45	236	26.7	87	98			
X3 LE	8.3	265	25.3	82	86			
X4 BE							Y	2012
X4 RE	6.54	367	12.7	69	56			
X4 LE	6.9	321	13.9	68	62			
X5 BE							N	
X5 RE	7.98	320	22.4	83	96			
X5 LE	8.3	276	27.6	89	103			
X6 BE							Y	1990
X6 RE	7.9	312	20.4	73	89			
X6 LE	8.1	267	24.2	79	92			
X7 BE							N	
X7 RE	7.34	301	25.5	87	87			
X7 LE	7.65	285	27.5	89	93			
X8 BE								

X8 RE	7.98	264	27.67	89	103			
X8 LE	6.5	324	12.6	65	72		Y	2007
X9 BE							N	
X9 RE	7.24	302	27.8	87	78			
X9 LE	8.1	267	25.3	82	93			
X10 BE							N	
X10 RE	8.4	224	28.7	92	110			
X10 LE	8.6	213	29.7	93	113			
X11 BE								
X11 RE	7.8	312	21.9	84	97		Y	1990
X11 LE	8.5	265	28.3	91	109			
X12 BE							N	
X12 RE	7.6	286	26.7	87	96			
X12 LE	7.9	265	28.1	91	97			
X13 BE							Y	1994
X13 RE	7.1	280	14	62	62			
X13 LE	7.2	234	13.8	56	56			
X14 BE							N	
X14 RE	9.1	167	26.1	86	86			
X14 LE	8.9	187	25.9	84	83			
X15 BE							N	
X15 RE	7.8	264	23.4	82	92			
X15 LE	7.6	256	24.2	84	95			
X16 BE							Y	1996
X16 RE	7.1	310	21.4	74	77			
X16 LE	7.5	302	23.3	76	76			
X17 BE							N	
X17 RE	8.1	243	27.9	87	98			
X17 LE	8.5	213	28.76	87	103			
X18 BE							N	
X18 RE	7.35	286	23.4	79	78			
X18 LE	7.54	310	24.7	81	82			
X19 BE							Y	2012
X19 RE	5.67	178	12.4	72	58			
X19 LE	6.5	156	13.6	75	67			
X20 BE							N	
X20 RE	8.2	267	26.5	87	103			
X20 LE	8.25	243	25.07	89	98			
X21 BE							Y	2013
X21 RE	6.3	143	12.8	69	69			
X21 LE	6.4	156	13.2	71	72			
X22 BE								
X22 RE	7.5	280	22	76	86		Y	2009
X22 LE	8.1	232	25.3	87	92			
X23 BE							N	
X23 RE	8.21	213	26.7	86	98			

X23 LE	7.69	296	25.5	79	95			
X24 BE							N	
X24 RE	8.97	145	31.2	88	103			
X24 LE	9.3	169	33.2	95	114			
X25 BE								
X25 RE	7.9	267	26.1	86	82			
X25 LE	7.23	287	21.6	76	76		Y	1990
X26 BE							N	
X26 RE	6.56	342	17.3	72	64			
X26 LE	7.21	289	22.3	79	72			
X27 BE							Y	2003
X27 RE	10.43	185	26.3	96	115			
X27 LE	10.01	135	25.7	93	103			
X28 BE							N	
X28 RE	10.02	134	29.5	94	92			
X28 LE	9.96	154	31.2	93	86			
X29 BE								
X29 RE	5.76	156	12	65	53		Y	2006
X29 LE	8.3	289	27.3	86	84			

LogMAR	2.50%	1.25%
50	33	22
45	33	22
30	11	0
60	13	24
50	18	24
55	45	46
55	34	20
55	25	0
55	30	14
51	45	45
30	20	25
50	30	45
57	60	9
30	0	0
57	22	9
55	6	0
50	6	0
55	14	0
41	34	25
41	30	0
34	28	25
60	30	25
60	30	25
45	20	15
60	40	40
60	40	30
55	30	25
40	3	0
30	0	0
25	0	0
51	25	15
49	25	15
32	10	11
45	16	6
45	14	1
42	1	2
60	43	35
55	29	30
50	25	25
60	45	30
60	39	24
55	38	23
60	35	25
55	30	9
55	30	4

50	24	15
45	20	5
40	10	1
51	35	35
44	25	4
48	30	10
50	40	30
50	24	20
50	25	10
55	40	20
55	30	2
50	25	2
55	41	35
50	39	20
50	41	31
50	40	40
50	40	40
50	40	40
50	30	30
40	14	9
20	0	0
55	35	30
50	30	20
54	34	25
50	25	10
45	15	0
50	25	10
45	25	10
32	20	10
45	20	10
35	20	8
25	15	6
32	18	5
35	40	15
25	35	15
32	40	10
50	35	25
54	30	15
45	29	12
43	35	25
42	35	24
43	32	25
60	25	9
58	24	10
23	3	0
43	13	8

35	13	8
42	12	8
50	35	10
45	32	10
43	35	8
45	25	10
15	3	1
43	22	10
22	1	0
12	1	0
21	1	0
35	6	1
35	3	0
35	6	1
56	34	24
55	35	21
55	35	21
37	15	12
36	13	13
36	15	12
5	0	0
3	0	0
4	0	0
60	45	35
58	43	35
59	43	36
45	22	16
32	21	16
43	22	14
3	0	0
4	0	0
2	0	0
60	45	35
58	46	36
54	43	35
54	10	3
55	8	1
53	4	1
54	25	4
23	5	0
52	20	3
45	26	18
32	20	17
32	22	18
22	3	0
12	0	0

22	3	0
54	45	35
53	43	35
54	45	35
21	3	2
12	3	1
22	1	1
21	4	1
17	2	1
14	2	0
54	11	5
53	11	5
51	11	5
43	3	1
12	0	0
42	2	1

This spreadsheet performs **Spearman rank correlation** on up to 1000 observations.
For more information, see http://udel.edu/~mcdonald/statspearman.html.
It comes with the frigatebird data from that page entered as an example.
To use the spreadsheet, replace the frigatebird data with your numbers.

If you have fewer than 11 observations, the P values from the equation are inaccurate.
In that case, the P-values are looked up in a table of critical values, and you should use this "P-val

COLOUR	LogMAR	rank of x	rank of y
35		15.5	
35	35	50	50.5
	35		50.5
36		15.5	
36	36	15.5	15.5
	36		15.5
32		73	
35	32		74
	35		50.5
35		50	
36	35	15.5	50.5
	36		15.5
28		85	
36	28	15.5	86
	36		15.5
36		15.5	
36	36	15.5	15.5
	36		15.5
35		50	
36	35	15.5	50.5
	36		15.5
35		50	
30	35	79.5	50.5
	30		80.5
35		50	
36	35		50.5
	36		15.5
4		97	
2	4	98	98
	2		99
33		70	
	33		71
32		73	
30	32	79.5	74

Spearman's rho:
degrees of freedom:
P-value:

	30		80.5
35		50	
35	35	50	50.5
	35		50.5
35		50	
36	35	15.5	50.5
	36		15.5
35		50	
35	35	50	50.5
	35		50.5
36		15.5	
36	36	15.5	15.5
	36		15.5
30		79.5	
35	30	50	80.5
	35		50.5
29		83	
31	29	76.5	84
	31		77.5
35		50	
35	35	50	50.5
	35		50.5
35		50	
35	35	50	50.5
	35		50.5
35		50	
36	35	15.5	50.5
	36		15.5
36		15.5	
36	36	15.5	15.5
	36		15.5
35		50	
36	35	15.5	50.5
	36		15.5
29		83	
35	29	50	84
	35		50.5
35		50	
36	35	15.5	50.5
	36		15.5
25		86.5	
22	25	93	87.5
	22		94
35		50	

36	35	15.5	50.5
	36		15.5
23		90.5	
23	23	90.5	91.5
	23		91.5
35		50	
35	35	50	50.5
	35		50.5
35		50	
21	35	95	50.5
	21		96
36		15.5	
36	36	15.5	15.5
	36		15.5
35		50	
36	35	15.5	50.5
	36		15.5
32		73	
35	32	50	74
	35		50.5
32		73	
30	32	79.5	74
	30		80.5
25		86.5	
24	25	88.5	87.5
	24		89.5
35		50	
35	35	50	50.5
	35		50.5
35		50	
35	35	50	50.5
	35		50.5
0		100	
1	0	99	101
	1		100
35		50	
35	35	50	50.5
	35		50.5
36		15.5	
35	36	50	15.5
	35		50.5
36		15.5	
36	36	15.5	15.5
	36		15.5

36		15.5	
36	36	15.5	15.5
	36		15.5
32		73	
29	32	83	74
	29		84
31		76.5	
35	31	50	77.5
	35		50.5
35		50	
36	35	15.5	50.5
	36		15.5
22		93	
19	22	96	94
	19		97
35		50	
36	35	15.5	50.5
	36		15.5
36		15.5	
36	36	15.5	15.5
	36		15.5
35		50	
35	35	50	50.5
	35		50.5
35		50	
36	35	15.5	50.5
	36		15.5
24		88.5	
22	24	93	89.5