A COMPARATIVE STUDY ON OPTIC NERVE FUNCTION, RETINAL NERVE FIBRE LAYER THICKNESS AND VEP PRE AND 3 MONTHS POST TREATMENT WITH ETHAMBUTOL IN TUBERCULOSIS PATIENTS

BY

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DISCLAIMER

I hereby certify that the work in this dissertation is my own except for quotations and summaries which have been duly acknowledged.

Dated: 23rd May 2017 …..……..……

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ABSTRAK

Latar belakang

Ethambutol merupakan antara rawatan utama bagi penyakit tuberkulosis. Walau bagaimanapun ubat ini berpotensi untuk menyebabkan kemerosotan fungsi penglihatan yang kekal. Kajian ini adalah untuk menunjukkan perbandingan di antara parameter anatomi dan fungsi penglihatan dengan menggunakan kaedah fungsi saraf optik secara konvensional dan dengan mengukur ketebalan lapisan gentian saraf retina serta corak potensi rangsangan saraf sebelum dan selepas rawatan kepada pesakit tuberkulosis yang di rawati menggunakan regim yang mengandungi ethambutol.

Kaedah

Ini ialah kajian prospektif yang melibatkan 72 biji mata seramai 36 orang pesakit yang telah dirawat dengan ethambutol melalui strategi Pemerhatian Langsung Rawatan Jangka Pendek di Hospital Universiti Sains Malaysia, Kelantan, Malaysia. Kadar ketepatan penglihatan, fungsi saraf optik, keluasan medan penglihatan, analisis lapisan gentian saraf retina (RNFL) menggunakan topografi kohoren optik (OCT) serta corak potensi rangsangan saraf (PVEP) telah dinilai. Pemeriksaan telah dijalankan sebelum terapi dimulakan serta pada tempoh 3 bulan setelah rawatan dimulakan.

Keputusan

Analisis keluasan medan penglihatan pada parameter purata lencongan (md) menunjukkan perubahan yang signifikan secara statistik ($p=0.010$). Terdapat juga perubahan yang signifikan pada OCT dan PVEP. Perbandingan ketebalan pada RNFL menunjukkan peningkatan ketebalan pada semua kuadran ($p<0.05$) dan kelewatan pada puncak pendam P100 serta pengurangan amplitud dalam penilaian PVEP ($p<0.001$). Tiada perbezaan antara kadar ketepatan penglihatan, kadar penglihatan warna, kadar pengamatan cahaya, kadar ketepuan warna merah serta keadaan fundus sebelum dan selepas rawatan dengan ethambutol.

Kesimpulan

Penggunaan OCT untuk mengesan ketebalan RNFL dan PVEP untuk mengukur amplitud serta kelewatan P100 boleh membantu dalam pengesanan perubahan pada struktur anatomi serta fungsi saraf optik lebih awal berbanding ujian fungsi saraf secara konvensional semasa di peringkat subklinikal. Perubahan ini mungkin disebabkan oleh keracunan ethambutol pada peringkat awal dan ini mementingkan penggunaan OCT dan PVEP penting bagi pemantauan pesakit tuberkulosis.

Abstract

Introduction

Ethambutol is a first line drug for the treatment of tuberculosis. However this drug has caused incidences of irreversible ocular toxicity. This study is to compare the anatomical and visual function using conventional optic nerve function tests, measurement of retinal nerve fibre layer and pattern visual evoked potential changes in patients with tuberculosis treated with a regime containing ethambutol.

Method

This is a prospective study involving 72 eyes of 36 patients treated with ethambutol according to the Directly Observed Treatment Short Course (DOTS) strategy in Hospital Universiti Sains Malaysia, Kelantan, Malaysia. The optic nerve function, retinal nerve fibre layer (RNFL) on optical coherence topography (OCT) and pattern visual evoked potential (PVEP) were assessed. The examination was performed before the start of therapy and three months after.

Results

Visual field analysis of mean deviation (md) showed significant statistical change ($p=0.010$). There were also significant changes on OCT and PVEP. Comparison of RNFL thickness showed an increased thickness in all quadrants ($p<0.05$) and a delayed P100 peak latency and decreased amplitude on PVEP assessment ($p<0.001$). There was no change in visual acuity, colour vision, light brightness, red saturation and fundus findings pre and post ethambutol.

Conclusion

The use of OCT to detect RNFL thickness and PVEP to assess P100 latency and amplitude can assist in the detection of subclinical anatomical and visual function changes prior to conventional optic nerve function tests. These changes may represent early ethambutol related optic neuropathy, making OCT and PVEP important tools in monitoring tuberculosis patients.

Chapter 1

Introduction

INTRODUCTION

Tuberculosis is an endemic disease, especially in developing countries like Malaysia. It is caused by *Mycobacterium tuberculosis*, which can infect any part of the body, but mainly infects the lungs.

Ethambutol hydrochloride is commonly used for the treatment of tuberculosis and *Mycobacterium avium* complex infections. Its exact mechanism of action is unknown; however the drug acts as a chelating agent by disrupting one of the several metal-containing enzymes in the nucleic acid structure of mycobacteria.

Ethambutol has been used as a first line drug in the treatment of tuberculosis, along with other drugs. It has been proven effective in combating *Mycobacterium tuberculosis.* However there is a drawback, as there is evidence that ethambutol causes toxic optic neuropathy.

The normal daily dose is 15mg/kg/day, which is also practiced in Hospital Universiti Sains Malaysia. Studies have shown that there is no safe dose for ethambutol. There have been recorded cases of ethambutol toxicity in cases where a lower dosage of the drug was used. There have been reports of toxicity occurring as early as immediately or one month after treatment. Thus it has become a practice for treating physicians to refer these patients to ophthalmologists prior to starting the drugs to obtain baseline ocular findings. This ensures that the future changes related to ethambutol toxicity can be detected.

Early detection of the disease may help in diagnosing and preventing occurrence of severe toxic optic neuropathy in the early stages of toxicity. The common practice is to examine and assess the optic nerve functions which include the vision, relative afferent pupillary defect, colour vision, red desaturation, light brightness and visual field changes.

Optical coherence topography (OCT) of the retinal nerve fibre layer (RNFL) and electrophysiological tests have the potential to document subtle anatomical and functional changes. OCT is an instrument that is used to measure the retinal thickness and its layers. It has now become an important tool to view the cross-sectional image of the microstructure of the retina. It is commonly used to measure the nerve fibre layer in glaucoma patients by accessing the optic disc parameters and also the RNFL to detect thinning of the normal retinal layers around the optic disc.

The OCT could prove to be a useful tool to detect early changes in RNFL thickness in patients post ethambutol treatment. However, studies regarding RNFL changes in ethambutol treatment have given varied results. These changes have been suggested to be attributed to ethambutol toxicity. The postulation is that ethambutol causes toxicity similar to alcohol induced toxicity. Ethambutol is derived from butanol. Ethambutol produces the metabolite ethylenediiminodibutyric acid, which is possibly involved in the transformation of metal ions to chelating agents. This leads to the chelation of copper ions within the optic nerve axons, causing decreased levels of copper. Due to this process, a disruption in axonal transport occurs. The disruption of axonal transport is suspected to cause energy depletion and axonal swelling in the

early stages. After the initial swelling, there is papilomacular-bundle-nerve necrosis, which leads to further neuronal-cell death, which manifests as thinning of the RNFL. These findings suggest that OCT of RNFL can detect early anatomical changes possibly due to ethambutol toxicity, which are not visible in a normal routine eye examination.

Visual Evoked Potential (VEP) is an electrophysiological test that records visually evoked electrophysiological signals extracted from the electroencephalograhic activity in the visual cortex recorded from the overlying scalp. As the visual cortex is activated primarily by the central visual field, VEP depends on the functional integrity of central vision at any level of the visual pathway including the eye, retina, optic nerve, optic radiations and occipital cortex. VEP may be able to detect anterior visual conduction disturbances and functional changes prior to gross neuro-ophthalmic examination abnormalities.

There are two methods of measuring VEP, depending on the patient's vision. Flash VEP is used for patients with poor vision who cannot focus on the "X" checkerboard stimulus. Pattern VEP is used on patients with good vision and who can focus on the stimulus. It also has greater sensitivity and accuracy.

VEP may be abnormal in patients with no visual pathway defect, such as amblyopia, uncorrected refractive error, media opacity and inattention or lethargy. Thus a complete examination and correction of refractive error has to be done prior to the test.

P100 is the most consistent and least variable peak. Delayed P100 peak latency and decreased amplitude are expected in cases with meningitis, optic neuritis or neuropathy, demyelination, anoxia, stroke and other possibilities of diseases that affect the retina, optic nerve or brain.

This study aims to evaluate the changes in optic nerve function, RNFL and pattern VEP pre and 3 months post treatment with ethambutol in tuberculosis patients. We postulate that these changes may be due to ethambutol ocular toxicity. We recruited patients with normal optic nerve function, OCT and VEP pre ethambutol, and assessed them again three months post treatment to look for changes in RNFL on OCT and VEP P100 latency and amplitude. We also assessed optic nerve function via conventional tests. We hope to discover whether OCT and VEP can detect changes that may signify subclinical optic nerve dysfunction in ethambutol treatment.

Chapter 2

Study Protocol

INTRODUCTION

2.1 OVERVIEW OF ETHAMBUTOL USAGE AND TOXICITY

Ethambutol hydrochloride is commonly used for the treatment of tuberculosis (TB) and *Mycobacterium avium* complex infections. Its exact mechanism is unknown; however the drug acts as a chelating agent by disrupting one of the several metal-containing enzymes in the nucleic acid structure of mycobacteria.[1]

Ethambutol is an effective drug in the first line treatment for tuberculosis. However its use has been associated with the incidences of ocular toxicity[2-3]. The incidence of ocular related ethambutol toxicity was noted to be from 1% to 18% from various studies.[4-5]

The most hazardous ocular side effect of this drug is toxic optic neuropathy which is dependent on the dose and duration of treatment. It has been reported that ethambutol results in bilateral optic neuropathy and includes both central and peripheral types.[6]

Characteristics of ocular toxicity of ethambutol

Classically the ocular toxicity is described as dose-related and duration-related,

Dose-related

There has been no reported 'safe-dose' of ethambutol.[7-8] Furthermore there have been no conclusive evidence correlating the severity of the neuritis of the optic nerve with the total intake of ethambutol.[9]

The usual daily dose is 15mg/kg/day[10-11] (that is also the routine dose in Hospital Universiti Sains Malaysia (HUSM)), though there have been cases of reported toxicity observed at doses as low as 12.3mg/kg per day.[12]

Duration-related

It has been reported that the mean interval between onset of therapy and the toxic effects is around 3 to 5 months.[7,12-13] On the other hand an article has noted quite a high prevalence of toxicity at 1 to 2 months of treatment and there is a report of immediate toxicity following ethambutol treatment.[12]

Reversibility

There are studies which found that patients who experience ethambutol toxicity often have severe and persistent visual defects, despite the fact that they have received the appropriate dosages and are monitored regularly for visual acuity and colour vision with prompt discontinuation of the drug, with the slightest sign of toxicity[9,14].

However, not all the patients who had described visual acuity improvement after the discontinuation of the drug had complete recovery. An example of this would be the study conducted by Tsai and Lee's [14], where 50% of the patients sustained permanent visual impairment without recovery.[12,15]

Monitoring and preventive measures

There are several international guidelines which have been published suggesting measures used for the prevention and early detection of ethambutol-induced ocular toxicity. [16-20]

Despite regular follow-ups and optic nerve function tests performed on all patients on ethambutol with the recommended dosage, there are still those who develop ethambutol toxicity. This makes it difficult to believe the norm of regular optic nerve function tests, which includes visual acuity, visual field, colour vision test, light brightness and red desaturation which has been used to detect ocular toxicity is actually effective in clinical practice.

Early detection of ocular toxic effects before occurrence of symptoms is of great value to prevent extensive optic nerve damage and furthermore allow the complete recovery of normal function.[21]

The VEP has proven very useful in detecting anterior visual conduction disturbances where there are little disturbances in the neuro-ophthalmological examination.[22-25]

Yiannikas[8], Van Lith[26] and Melamud[27] found that VEP may be considerably disturbed at a stage when there is little neuro-ophthalmologic examination abnormality.

An essential practice during routine eye follow-ups for patients on ethambutol therapy is to assess their optic nerve function while they are on the medication and 6 months post cessation of ethambutol.

Optic nerve functions consist of relative afferent pupillary defect examination, colour vision, red desaturation, light brightness and visual field. Relative afferent pupillary defect is examined in a dark room using a bright torch. Patients are instructed to look at a distance and not at the torch. The torch is swung from one eye to the other and the pupil is observed to note the constriction of the pupil size.

Colour vision is assessed using Ishihara plates which detect red-green colour defects. The plates consist of a number of coloured plates that have randomised dots which differ in colour and size. These patterns of dots form a number or shape clearly visible to subjects with normal colour vision but invisible or difficult to see for subjects who have red-green colour vision defect. There are certain plates that can produce or transform a figure that can only be seen by colour deficient individuals. The scoring depends on the number of plates that the patient is able to interpret correctly.

Red desaturation and light brightness is done by subjectively asking the patient to compare a bright red object and a bright torch light separately by accessing one eye at a time. Generally red desaturation is examined first then light brightness. The patient has to compare if the red colour hue and light brightness is the same in either eye or reduced in one compared to the other. The extent of deficiency is amounted in percentage.

Visual field is recorded using the Humphrey Visual Field. The desired field is 24-2. This machine assesses one eye at a time, 24 degrees of the patient's field of view is tested by instructing the patient to focus on a centre point in the dome of the Humphrey Visual Field while flashing lights will appear randomly around. If the patient is able to see the flashing lights without moving his/her eye, the patient is required to press a button which is given to him/her. A printout of the patient's result will be used to interpret if there is any visual field loss. Patients with optic neuropathy usually will present with enlarged blind spots or paracentral scotomas.

RNFL thickness is accessed using OCT. Each eye is tested one at a time. The patient is asked to look straight at a green light in the machine without blinking or moving and measurements of the optic nerve head parameters are taken and analysed by the OCT machine. The RNFL thickness is measured in the peripapillary region surrounding the optic disc. It is divided into four quadrants namely the superior, inferior, temporal and nasal quadrant. The average retinal nerve fibre is measured using spectralis OCT (Hyun JS et al, 2011) Average thickness 106.38 um, superior 125.00 um, inferior 132.46 um, temporal 91.04 um, nasal 78.50 um.

OCT

OCT has now become an important tool to view the cross-sectional image of the microstructure of the retina. It is commonly used to measure the nerve fibre layer in glaucoma patients [28]. The OCT could prove to be a useful tool to detect early changes in RNFL in patients post ethambutol treatment. In a study that was performed by Samantha J Chai et al. and Zoumalan CI et al., their results showed that there was a decrease in the RNFL thickness especially in the temporal quadrant

in patients who developed ethambutol induced optic neuropathy.[29-30] However there was one study done by Jae Keun Chung et al that found an increase in average retinal nerve fibre thickness.[31]

In this study we expect to find a decreased retinal nerve fibre thickness especially in the temporal quadrant of the optic nerve head.

In 1991, Huang et al[32] reported the OCT, was used as a non- invasive ocular imaging tomography. It uses a coloured representation of the tissue structures, based on the intensity of the returned light. OCT has been used to measure the retinal nerve fibre thickness of the optic nerve head and macula. It has been used to measure and monitor the retinal nerve fibre thickness in glaucoma and macula diseases patients. OCT uses light to measure micrometer axial resolution.

OCT uses a low coherence inferometry as its basic principle, where the light source is split into two, one that goes into the eye and the other to the reference path. The light reflected back from the two paths forms an interference pattern. Due to its coherent detection, OCT measures reflecting retinal layers and its thickness. Augmentation of these reflecting signals make it able to construct a retinal thickness map and to determine the presence of any decrease or increase in thickness.[33]This makes it an important tool in evaluating retinal nerve fibre thickness.[34]

Visual Evoked Potentials

The VEP is an important electrophysiological test in the investigation of suspected optic nerve disease. Electrical potential differences recorded from the scalp in

response to light or pattern stimulation of the eye will be able to detect functional loss in the visual pathway from retina to the visual cortex. The VEP is very useful in detecting anterior conduction disturbances; but it is not specific with regards to the aetiology.

VEPs have been described by Odom et al. (2009)[35]. They are caused by sensory stimulation of the subject's visual field and are observed using electroencephalography.

VEPs are visually evoked electrophysiological signals extracted from the electroencephalograhic activity in the visual cortex recorded from the overlying scalp. As visual cortex is activated primarily by the central visual field, VEPs depend on the functional integrity of central vision at any level of the visual pathway including the eye, retina, optic nerve, optic radiations and occipital cortex.

The waveform of a VEP depends on the temporal frequency of the stimulus. At rapid rates of stimulation, the waveform becomes approximately sinusoidal and is termed 'steady state'. At low temporal frequencies, the waveforms consist of a number of discrete deflections and are termed transient VEP. All International Society for Clinical Electrophysiology of Vision (ISCEV) standard VEPs are transient.

The standard presents minimum protocols for basic clinical VEP recording. Three standard stimulus protocols are defined. The ISCEV standard VEP protocols are defined for a single recording channel with a midline occipital active electrode. If chiasmal or retrochiasmal disease is suspected, a three-channel montage, using the

midline and two lateral electrodes, is recommended in addition to the basic standard tests. Following a principle established in earlier standards, ISCEV has selected a subset of stimulus and recording conditions, which provides core clinical information that can be performed by most clinical electrophysiology laboratories throughout the world. These are:

Pattern-reversal VEPs elicited by checkerboard stimuli with large 1_ (60 min of arc) and small 0.25_ (15 min) checks.
Pattern onset/offset VEPs elicited by checkerboard stimuli with large 1_ (60 min of arc) and small 0.25_ (15 min) checks.
Flash VEP elicited by a brief luminance increment, a flash, which subtends a visual field of at least 20.

Pattern reversal is the preferred stimulus for most clinical purposes. Pattern-reversal VEPs are less variable in waveform and timing then the VEPs elicited by other stimuli. The pattern onset/offset is best suited for the detection of malingering and for the use in patients with nystagmus. Flash VEP are useful when poor optics, poor cooperation or poor vision makes the use of pattern stimulation inappropriate. To comply with this standard, at least one standard protocol should be included in every clinical VEP recording session so that all laboratories will have a common core of information that can be shared or compared.[35]

ISCEV recognizes that the VEPs may be elicited by a wide range of stimulus protocols that are not covered in the standard. Some are widely used in specialised VEPs and extended VEP protocols.

Manufacturers are encouraged to produce equipment that can perform as many of these specialised tests as possible. By limiting these standards to three protocols, the intension is that standard VEPs will be incorporated universally into clinical VEP testing along with the additional tests and extended protocols that a laboratory may choose to use. The standard does not require that all three protocols should be used for every investigation on every patient.

VEP pattern reversal checkerboard will be used in conducting this study. The pattern reversal VEP consists of a prominent positive component at approximately 100ms (P100) preceded and followed by negative components (N75 and N135).

In ethambutol induced optic neuropathy, it is expected to have an abnormal optic nerve function, reduction in retinal nerve fiber thickness in OCT of optic nerve head and prolonged P100 latency and reduced P100 amplitude in VEP.

2.2 RATIONALE OF THIS STUDY

In ethambutol toxicity, an accurate detection of toxicity is crucial for the diagnosis. Some doctors have reported sudden and irreversible loss of vision in ethambutol treated patients. The VEP has been known to be a very useful tool in detecting an anterior visual conduction disturbance when there is minimal disturbance in the neuro-ophthalmological examinations.[21] There have been a few studies on tuberculosis patients, where it has been found that VEP and OCT have the ability to detect changes which were not detected on routine examination of visual function.[8,15,21,29-30] These findings confirm the usefulness of VEP and OCT in the

detection of subclinical optic nerve disease and suggest their use in routine monitoring of ocular function in patients treated with ethambutol.

The aim of this study is to compare the optic nerve function, RNFL thickness using OCT and VEP changes prior to starting ethambutol and after 3 months of treatment. This study may provide information on retinal nerve fiber thickness using OCT and VEP as a potential tool in detecting early changes in ethambutol induced optic neuropathy. It can also monitor disease progression in tuberculosis patients treated in HUSM.

2.3 OBJECTIVES

2.3.1 GENERAL OBJECTIVES

To compare optic nerve functions, RNFL thickness and VEP (P100 latency and amplitude) at pre and 3 months post treatment with ethambutol in tuberculosis patients.

2.3.2 SPECIFIC OBJECTIVES

1) To compare optic nerve functions at pre and 3 months post treatment with ethambutol in tuberculosis patients.

2) To compare RNFL thickness at pre and 3 months post treatment with ethambutol in tuberculosis patients.

3) To compare VEP changes (P100 Latency and Amplitude) at pre and 3 months post treatment with ethambutol in tuberculosis patients.

2.3.3 RESEARCH QUESTIONS

1) Is there a significant change in optic nerve functions at pre and 3 months post treatment with ethambutol in tuberculosis patients?

2) Is there a significant change in RNFL thickness at pre and 3 months post treatment with ethambutol in tuberculosis patients?

3) Is there a significant change in VEP changes (P100 Latency and Amplitude) at pre and 3 months post treatment with ethambutol in tuberculosis patients?

2.3.4 HYPOTHESIS

There is a significant change in optic nerve functions, RNFL thickness in OCT (decrease in thickness) and in VEP (P100 latency and amplitude) at 3 months after starting ethambutol in tuberculosis patients.

2.4 METHODOLOGY

2.4.1 RESEARCH DESIGN

Prospective study, one group pre intervention- post intervention design.

2.4.2 STUDY SETTING AND PERIOD

Venue : Ophthalmology Clinic, HUSM.

Duration : August 2013- May 2015

2.4.3 REFERENCES AND STUDY POPULATION

Target : Patients diagnosed with tuberculosis in Malaysia

Source : Patients diagnosed with tuberculosis in HUSM

Sampling frame : Patients diagnosed with tuberculosis in HUSM from August 2013 until May 2015 that fits into inclusion criteria. Every third patient on the list of referred patient is selected.

2.4.4 SAMPLING METHOD

Simple random sampling is used. Every third patient on the list of referred patient is selected.

2.4.5 SELECTION CRITERIA

2.4.5.1 INCLUSION CRITERIA

- Patients with pulmonary or extra-pulmonary tuberculosis.
- Aged above 18 years.
- Patients on ethambutol containing regime.

2.4.5.2 EXCLUSION CRITERIA

- Patients with tubercular meningitis.
- Patients with cerebral tuberculosis.
- Patients with renal impairment.
- Patients with past history of anti-tubercular therapy.
- Mentally challenged patients.
- Patients with developmental brain disease.
- Patients with active brain disease.

- Patients who do not consent.
- Patients below 18 years.
- Patients with abnormal VEP/RNFL on first time evaluation before treatment.
- Patients with corneal opacity or presence of dense ocular media opacity.

2.5 SAMPLING AND SAMPLE SIZE

Sample Size Calculations

To compare optic nerve functions, RNFL thickness and VEP (P100 latency and amplitude) at pre and 3 months post treatment with ethambutol in tuberculosis patients.

Sample size calculation done by using STATA software for one sample with repeated measures.

(*VEP latency is more sensitive and accurate than amplitude to detect ethambutol toxicity,*[1,9,15,21,22,31]*also we did not find any mean for amplitude in the literature reviews, so the use of mean and SD latency for sample size calculations.)*

P100 Latency

sampsi 0 2.5, sd1(5.02) alpha(.05) power(.8) pre(1) post(1) r01(.5) one sample

Estimated sample size for one sample with repeated measures

Assumptions:

alpha = 0.0500 (two-sided)

power = 0.8000

alternative m = 2.5 (Jae Keun Chung et al 2012)

sd = 5.02 (Jae Keun Chung et al 2012)

number of follow-up measurements = 1

number of baseline measurements = 1

correlation between baseline & follow-up = 0.500

Method: CHANGE

relative efficiency = 1.000

adjustment to sd = 1.000

adjusted sd1 = 5.020

Estimated required sample sizes:

n1 = 32

(*OCT is used to measure the RNFL thickness to detect changes in thickness in ethambutol toxicity.*[29-31]*)*

RNFL Average

sampsi 0 4.9, sd1(9.8) alpha(.05) power(.8) pre(1) post(1) r01(.5) one sample

Estimated sample size for one sample with repeated measures

Assumptions:

alpha = 0.0500 (two-sided)

power = 0.8000

alternative m = 4.9 (Jae Keun Chung et al 2012)

sd = 9.8 (Jae Keun Chung et al 2012)

number of follow-up measurements = 1

number of baseline measurements = 1

correlation between baseline & follow-up = 0.500

Method: CHANGE

relative efficiency = 1.000

adjustment to sd = 1.000

adjusted sd1 = 9.800

Estimated required sample sizes:

n1 = 32

The largest n= 32

We did not find any mean for optic nerve function in the literature reviews, so the use of mean and SD latency for sample size calculations were taken according to the highest n obtained from available resources.

Sample size calculation for paired t-test analysis

Including dropout + 10%

n = 32/(1-0.1) = 32/0.9 = 36 patients (72 eyes). Both eyes evaluated as the changes may be asymmetrical.

2.6 DEFINITION OF TERMS

2.6.1 VEP

Visual Evoked Potential is the evoked potential or evoked response used to determine the visual function of a person or animal. It uses an electrical potential that is recorded after presenting a stimulus that stimulates the visual field, which is detected by electrophysiological recording methods. Subjects can be stimulated using flashing lights or checkerboards that flicker between black on white or white on black.

2.6.2 SNELLEN CHART

A Snellen chart is a chart which is used to measure the visual acuity of a person. It was created by a Dutch ophthalmologist in 1862 named Herman Snellen. The Snellen chart is printed with eleven lines of block letters, which progressively decrease in size. Visual acuity is measured from 6 meters away and can be performed in subjects who cannot identify letters or numbers using the E chart.

2.6.3 SLIT LAMP

A slit lamp is an instrument consisting of multiple telescope lenses with a high intensity light source that is used to view a person's eye. It can be used to view the anterior and posterior segments of the eye. It provides a stereoscopic magnified view of all the eye structures in conjunction with other external lens devices.

2.6.4 HUMPHREY VISUAL FIELD

The Humphrey Visual Field is an instrument used to test a subject's visual field up to 30 degrees. The subject has to be able to sit up for this test. The patient is set in front of a small concave dome in a small machine with a target in the centre and the patient is rested on a chin rest and focuses on the centre target. Lights are shone inside the dome randomly and the subject will press on a button when he/she sees the light. It is used for detection of glaucoma, certain neurological conditions and conditions affecting the optic nerve.

2.6.5 ISHIHARA PLATES

Ishihara colour test plates are used to test colour vision especially for red-green colour deficiency. It was created by Dr. Shinobu Ishihara in 1917. This test consists of a number of coloured plates which contain a circle of dots that are random in size and colour. These patterns of dots form a number or shape clearly visible to subjects with normal colour vision but invisible or difficult to see for subjects that have red-green colour vision defect. There are certain plates that can produce or transform a figure that can only be seen by colour deficient individuals.

2.6.6 FUNDUS PHOTOGRAPH

A fundus photograph is done using a machine made specifically to take a picture of the posterior segment of the eye. It is used to keep an accurate record of the optic nerve, macula, vessels and vitreous of a subject's eye. The patient is seated and is asked to look directly at the light inside the machine and a photograph is taken.

2.6.7 VISUAL ACUITY

Visual acuity is the measurement of spacial resolution and acuteness or clearness of vision. It is tested using charts like the Snellen chart. Normal visual acuity is 6/6 or 20/20. Visual acuity can be corrected by refraction if it is due to refractive error. It is important to have normal ocular and brain function to have normal visual acuity.

2.6.8 COLOUR VISION

Colour vision is the ability to perceive colours accurately, which is based on the wavelength of light. Perception of colours is a subjective process where the brain responds to the stimuli that is produced by how light reacts with cone photoreceptors.

2.6.9 VISUAL FIELD

Visual field is the total object in peripheral vision that can be seen by a subject while the subject focuses his/her eye centrally. The visual field test can detect dysfunction in central or peripheral vision. It can be tested by comparing the subject's field of vision with the examiner's or using perimetry.

2.6.10 REFRACTION

Refraction uses a series of test lenses to measure a subject's refractive error. Refractive error is tested with a patient sitting down while focusing on an object six metres away, as an examiner uses a retinoscope to see the reflection in the eye and attempts to neutralise the reflection using test lenses. Refractive error is corrected using glasses or contact lenses.