# EVALUATION OF HEMIFIELD SECTOR ANALYSIS PROTOCOL IN MULTIFOCAL VISUAL EVOKED POTENTIAL (MFVEP) OBJECTIVE PERIMETRY FOR THE DIAGNOSIS AND EARLY DETECTION OF GLAUCOMATOUS FIELD DEFECTS

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**SUMMARY**
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**INTRODUCTION:** Visual field assessment is a core component of glaucoma diagnosis and monitoring, and the Standard Automated Perimetry (SAP) test is considered up until this moment, the gold standard of visual field assessment. Although SAP is a subjective assessment and has many pitfalls, it is being constantly used in the diagnosis of visual field loss in glaucoma. Multifocal visual evoked potential (mfVEP) is a newly introduced method used for visual field assessment objectively. Several analysis protocols have been tested to identify early visual field losses in glaucoma patients using the mfVEP technique, some were successful in detection of field defects, which were comparable to the standard SAP visual field assessment, and others were not very informative and needed more adjustment and research work. In this study, we implemented a novel analysis approach and evaluated its validity and whether it could be used effectively for early detection of visual field defects in glaucoma. **OBJECTIVES:** The purpose of this study is to examine the effectiveness of a new analysis method in the Multi-Focal Visual Evoked Potential (mfVEP) when it is used for the objective assessment of the visual field in glaucoma patients, compared to the gold standard technique. **METHODS:** 3 groups were tested in this study; normal controls (38 eyes), glaucoma patients (36 eyes) and glaucoma suspect patients (38 eyes). All subjects had a two standard Humphrey visual field HFA test 24-2 and a single mfVEP test undertaken in one session. Analysis of the mfVEP results was done using the new analysis protocol; the Hemifield Sector Analysis HSA protocol. Analysis of the HFA was done using the standard grading system. **RESULTS:** Analysis of mfVEP results showed that there was a statistically significant difference between the 3 groups in the mean signal to noise ratio SNR (ANOVA $p<0.001$ with a 95% CI). The difference between superior and inferior hemispheres in all subjects were all statistically significant in the glaucoma patient group 11/11 sectors (t-test $p<0.001$), partially significant 5/11 (t-test $p<0.01$) and no statistical difference between most sectors in normal group (only 1/11 was significant) (t-test $p<0.9$). sensitivity and specificity of the HAS protocol in detecting glaucoma was 97% and 86% respectively, while for glaucoma suspect were 89% and 79%. **DISCUSSION:** The results showed that the new analysis protocol was able to confirm already existing field defects detected by standard HFA, was able to differentiate between the 3 study groups with a clear distinction between normal and patients with suspected glaucoma; however the distinction between normal and glaucoma patients was especially clear and significant. **CONCLUSION:** The new HSA protocol used in the mfVEP testing can be used to detect glaucomatous visual field defects in both glaucoma and glaucoma suspect patient. Using this protocol can provide information about focal visual field differences across the horizontal midline, which can be utilized to differentiate between glaucoma and normal subjects. Sensitivity and specificity of the mfVEP test showed very promising results and correlated with other anatomical changes in glaucoma field loss.

Key words: Objective perimetry, multifocal VEP, visual field testing, glaucomatous field loss, glaucoma suspect, SAP, HFA

**ACKNOWLEDGMENT**

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# 1.0 INTRODUCTION

## 1.1 BACKGROUND

The early diagnosis and effective monitoring of glaucoma are considered the major concerns and objectives, in almost all of the research studies conducted in the field of glaucoma management. Visual field assessment is still the main monitoring tool that reflects progressive glaucomatous functional loss. It has been reported [1,2] that at least 25-50% of the retinal ganglion cell axons must be lost prior to the development of a field abnormality detectable with modern automated visual field testing. However, newer techniques of visual field assessment have shown higher accuracy and detectability of these visual field defects, and currently are able to localize early focal visual field defects at an earlier stage, either directly through functional assessment or indirectly through morphometric assessment of the optic nerve measurements [3,5]. Thus, early detection from a clinical point of view means identifying those individuals who are undergoing unnoticed (with no visual field changes) serious ganglion cell death. Despite its valuable contribution to the management of glaucoma, standard perimetry is considered a process that has too many inputs; related to the operator, patient and test protocol, which could negatively influence the accuracy of the final outcome. The introduction of Standard Automated Perimetry (SAP) has improved the accuracy and reduced variability errors compared to earlier kinetic perimeters. The rationale behind this clinical research was the interest in developing a better method for objective visual field assessment, able to detect glaucomatous field defects at an early stage. Back in 2009 I was performing a standard multifocal visual evoked potential test (mfVEP) on a glaucoma patient, and accidently discovered an error in the algorithm of amplitude detection and how the markers were being allocated in the waveform. This error was a good motivation for me to explore the topic and develop a method of improving the detection of signal amplitude in the standard mfVEP waveform. In the literature, I found a novel method for detection of waveform components by allocating a signal and noise window, called the signal to noise ratio (SNR). The SNR incorporated the level of noise in each individual waveform when calculating the signal amplitude. This methodology has been extensively explored in the literature and has shown good clinical application. When I contacted the Roland Consult electrophysiology equipment manufacturer, Brandenburg- Germany, I explained to them my concerns and how the software should be upgraded to the use SNR analysis. They acknowledged the error in marker allocation in the waveform and informed me that they were in the process of upgrading and installing a newer version. I found that the two most commonly used methods for mfVEP data analysis were: comparison of the tested results to a set of normative data, or an inter-eye analysis, comparing one eye's data to the other. I had some concerns about the use of normative data; as mfVEP is a very variable test with wide range of normal response, for reasons discussed in the following sections. In addition, if both eyes are equally and significantly affected by glaucomatous visual field

defects the inter-eye analysis will not give any valid information. My idea of comparing specific parts of one hemifield to their fellow identical parts in the opposite hemifield is supported anatomically by the retinal nerve fibre layer (RNFL) following an identical distribution, and arcuate configuration, around the centre and respecting the midline. Physiologically, glaucomatous visual field defects usually starts as a focal loss of fibres in one sector in one hemifield before the contralateral hemifield is affected. Theoretically, if detection of early glaucomatous visual field defects is to be a major objective, then the optimal approach is to compare individual sectors and hemi-rings in one hemifield to their corresponding fellows in the opposite hemifield. With the known and documented high sensitivity of mfVEP test, this comparison could provide information about early focal depression in the visual field, which can reflect an early glaucomatous visual field defect. Roland Consult agreed to test this new analysis protocol and agreed to assist in the development of custom software for the purpose of this clinical research, testing the effectiveness of this protocol in detecting glaucomatous visual field defects by modification of the analysis protocol. The preparation of this software passed through 6 beta versions until it was finally prepared in its most suitable parameters, in early 2012. Standard stimulus parameters were used, as recommended by the International Society of Clinical Electrophysiology of Vision (ISCEV) using the data analysis protocol created for this research. The initial assumption was that this new protocol would be able to identify normal subjects as having normal visual fields and patients with glaucoma as having glaucomatous visual fields. The hope was that it could offer greater sensitivity in the assessment of glaucoma suspects and suspicious glaucoma presentations. The total preparation and work time devoted to this clinical research was 3 years; between 2010 and 2013.

### 1.1.1 The field of vision

The field of vision is defined as the area that is perceived simultaneously by the fixating eye. The normal limits of the field of vision are approximately $60^{o}$ superiorly, $75^{o}$ inferiorly, $110^{o}$ temporally and $60^{o}$ nasally [(3)]. The visual field defects can be monocular or binocular, respecting the horizontal or vertical midline, absolute or relatively less sensitive more than other parts of the field, scattered or following a specific pattern. Each type of defect represents a separate entity of pathological findings and reflects the location and severity of existing pathology. There are many types of optic neuropathies that can produce visual field defects of variable degrees and extensions; glaucoma is one of the most common causes of optic neuropathies among middle and old age groups worldwide. The glaucomatous visual field loss is characteristic to the disease in most of the presentations; in its locations, pattern of progression, and the clinical picture associated with. The detection of visual field defects is essential in clinical practice: as it can help determining the type of pathology involved and also it offers a reliable tool to monitor its progression. There are

important reasons as why visual field testing is needed. Firstly, visual field defects can be detected in certain locations and patterns, which can point to an existing pathology most commonly following a unique way of progression such as the pathology involved in glaucomatous defects and neurological visual field defects. Peripheral versus central field defects tend to give more specific information about the underlying pathology; as glaucomatous defects most commonly start peripherally and encroach on the central field gradually as the disease progresses, whilst other pathological processes such as optic neuritis can damage the central field. However, glaucomatous visual field defect can arise primarily from the central 5-10 degrees, especially when the temporal nerve fibre layer is affects such as in normal tension glaucoma, and it will be presented as a severe central visual field loss. Glaucoma is the most common of these types of disorders. Secondly, patients are often unaware of their peripheral vision loss, especially if it has progressed gradually and over a long period of time. Thirdly, some forms of optic neuropathies are reversible, while the patient can notice the vision defect during the disease; the clinician can monitor and measure the improvement of visual field by performing these tests on a regular basis [4,5].

### 1.1.2 Objective and subjective visual field assessment

The concept of subjective versus objective visual field assessment has been a source of debate in many studies. The term subjective: refers to the standard form of visual field testing using the current Humphrey Field Analyser (HFA) or any other equipment that runs SAP testing protocols. The subjectivity comes from the major dependency of the accuracy of the results on the patient's responses and answers to the test questions. A common cause of error usually related to this type of testing results from the variability of the patient's responses during one test (intra-test) or between tests (inter-test), the reliability of these responses and how accurately it provides a true mapping of the patient visual field [9,10]. There are some intrinsic factors related to the effect of glaucoma on local ganglion cells and its progression that increase the level of both intra-test and inter-test variability. Most importantly is the effect of focal scotomas on the adjacent normal ganglion cells; as the disease progresses, the edges of isolated scotomas and encroaching arcuate defects will fluctuate significantly and give rise to high rate of variable responses. This problem will be worsened when fixation losses increases as the disease progresses, that is why SAP protocols tend to give high variability as glaucomatous visual field defects progress [11,12,16]. On the other hand, objective visual field testing refers to these tests that offer credible assessment of the visual field without the need for patient's responses. There are two main types of objective testing; functional and anatomical (structural):

The anatomical/structural test usually gives an indirect assessment of visual field defects through measurement of ganglion cell thickness or retinal nerve fibre thickness around the optic disc.

Many testing machines and strategies are currently offering this anatomical assessment such as optical coherence tomography (OCT) and the confocal scanning laser ophthalmoscope (CSLO).

The objective functional visual field assessment offers an objective measurement of patient's response to certain light and pattern stimulus projected onto the retina. An example of this type of test is the multifocal electrophysiology test, which produces a topographical map of the field of vision showing and highlighting areas of defects and others with normal responses.

### 1.1.3 Problems with SAP in Visual Field Assessment

It has been well established that conventional visual field testing using SAP protocols are almost always influenced by intra and inter-test variability due to factors related to the test-taking condition, patient's responses and most importantly the nature of the disease and the influence of visual field scotomas on the intra-test fluctuation level in patient's responses, which is considered as a source of variability unrelated to the visual field examination, but to the way the shape of glaucomatous visual field defects influence retinal sensitivity, particularly at the borders of scotomas which ultimately influences how the patient responds [(11)]. Inter and intra-test variability, reliability, reproducibility and learning effect, are major problems in current visual field testing protocols. Almost all perimetry software and testing strategies have attempted to overcome some or all of these problems with various degrees of accuracy and success. In current practice the HFA is considered as the gold standard method for visual field assessment in clinical practice and glaucoma related clinical trials [(1,12)]. The Humphrey Field Analyser has been widely regarded and considered as the gold standard for automated visual field assessment for more than two decades. In addition to its wide use in day to day practice by clinicians, all major glaucoma clinical trials have used this system in perimetry assessment; including the Advanced Glaucoma Intervention Study (AGIS), the Collaborative Initial Glaucoma Treatment Study (CIGTS), the Early Manifest Glaucoma Trial (EMGT), the Normal Tension Glaucoma Study (NTGS), and the Ocular Hypertension Treatment Study (OHTS) [(35-38)]. However, the full-threshold visual field test is currently regarded as a quasi-standard in day-to-day perimetry testing [(3)]. Traditional and conventional methods for visual field testing are dependent on the subject's personal and intellectual factors; the level of cooperation, comprehension and accuracy of responses are all determinant factors that shape the reliability of SAP. For these reasons standard automated perimetry is somewhat difficult for both young children and elderly subjects [(13)]. The automated suprathreshold is another visual field test that is mostly used for screening. Its use still limited to screening of normal subjects, and to a lesser degree for those with suspicious risk factors for glaucoma. It is time consuming, and, particularly at damaged field locations, its threshold estimates are highly variable. The Swedish Interactive Threshold Algorithm (SITA) standard offers much shorter test times, but its variability is almost

similar to that of the full-threshold strategy [9,10,14,15]. Threshold tests are demanding procedures and many patients produce consistent results only after some training, which is not easy to achieve for all patients. Within day-to-day practice, any clinical application, calls for rapid, simple, and reliable visual field tests that can be performed by patients with no significant learning or training tasks required from the patient, and to provide the clinician with credible results at the same time. Thus when the SITA standard test was designed initially to reduce test-taking time, the inter and intra-test variability did not improve significantly with its use, as the test-retest variability of SITA is only marginally better than Full Threshold (FT), which is due to the intrinsic factors related to neuronal responses and significant fluctuations in sensitivity at focal scotomas [12,16,17]. To more accurately detect glaucomatous visual field defect and its progression, test strategies that provide repeatable threshold estimates in damaged locations are necessary [18].

## 1.2 THE STANDARD VISUAL FIELD TEST

### 1.2.1 History of perimetry

The assessment of visual field loss or defect passed through many renovations and improvements since its introduction in clinical medicine in 1856 when Von Graefe [20] used a chalk board and a piece of chalk to map the scotomas and the physiologic blind spot. His trial involved identifying to limited degree, field constrictions and hemianopias. He performed the first type of kinetic perimetry calling it campimetry. He described this method as flat surface perimetry, mapping only the central field defects, as the patient was asked to respond when he/she saw a light target emerge from a dimmer background. In 1857 Aubert and Forster [21] introduced a new tool, it was called the arc perimeter because it used an arc-shaped arm enabled the extreme limits of the visual field thus offering more degrees for investigation. This was followed 3 decades (1889) later by the work of Bjerrum and Rønne [22] who presented the concept of isopters and arcuate scotomas based on the unique anatomical distribution of the retinal nerve fibre layer. They performed “quantitative isopter assessment” of the visual field and mapped the prototypic scotoma of glaucoma and the arcuate defect that breaks out from the blind spot and bears his name until this moment. The contemporary form of visual field perimetry did not take its well established form till 1945 when Goldmann [23,24] introduced a new concept representing the field of vision to the patient in a testing bowl. He used a single light source with few mirrors and light filters to introduce the stimulus and the illumination (later the colour as well) of the background. The combination of the light intensity and background illumination produced a very good stimulus in a controlled form that can offer measurable responses. The concept of measuring the response to differential light intensities was the new era of standardized perimetry, where the test could be repeated, reported and interpreted in a set of standardized parameters. Due to its ability to

introduce a standardized form of visual field assessment, the Goldmann perimetry became the standard visual field test. The test showed many revisions in the years after, improving the strategies and aiming to make visual field mapping more accurate and precise. Based on the Goldmann model, many research work took place; most importantly the work of Fankhauser [25], Heijl [26], Krakau [27] and Lynn [28] in the 1970s. This decade had shown very active research work that laid the fundamental concepts of modern differential light sensitivity and the introduction of automated perimetry using the Goldmann perimetry bowl. Later in the mid 1980's been the introduction of the Octopus 201 perimeter manufactured by Haag Streit, Switzerland. Shortly after, the earliest version of the Humphrey field analyser series was manufactured by Humphrey instruments, CA – USA. The superiority of the Humphrey machine was evident from its early days due to its ease of use and high ability of standardization and since then it became the visual field testing industry standard. The Humphrey instrument brought some significant advantages. Although rendering of defect-shape is coarse because of the spacing of stimuli by 6° proved that static perimetry was more sensitive than older kinetic perimetry testing. The reasons for this preference were the standardization and accurate quantification of tests parameters, which makes it reproducible. The limitations faced by the non-automated instruments like training and learning curves were much improved by the use of automated version [29]. Over the years and with the continuous use of the new Humphrey machine, it became evident through much research work that the new automated test needs more attention in the patient instruction and dealing with the reliability of the test, which was influenced by its subjectivity nature and strong relation to direct patient responses in mapping the field of vision [30-32]. The lengthy standard tests became a source of poor reliability of test-takers, which was a good reason for research work to take place trying to improve testing algorithms making it shorter and more reliable. The implementation of SITA has halved the test time, which reached at the earlier static standard versions 15-20 minutes, and to a good extent improved its reliability and confidence intervals [33]. The SITA method uses in its algorithm a model of two maximum likelihood visual field responses; the glaucoma and normal patterns. The likelihood of a set of data is the probability of obtaining that particular set of data, given a chosen probability distribution model (in this case either a normal or glaucomatous model). The way these models were designed is based on a priori criteria such as the slope of frequency-of-seeing curves, which reflects variability, increasing with level of threshold [33,34].

### 1.2.2 Visual field protocols

As mentioned earlier, the ‘‘clinical standard’’ for detecting and monitoring glaucoma is static automated achromatic perimetry, Humphrey Field Analyser (HFA), which was introduced to the ophthalmology clinic in the early 1980s. The subject’s task is to press a button when he or she detects the presence of a small circular white stimulus (Goldmann Size III, 0.431 degree), of 200 milliseconds duration presented against a white background (10 cd / $m^2$). During the test patients are asked to fixate a central target, which usually available in different sizes and shapes to suit different levels of central visual acuity. For the 24-2 program, the test light is randomly presented to 54 locations out to an eccentricity 24 degrees of the visual field. These locations are regularly spaced 6 degrees apart. The tester has the option of including a test spot in the centre (fovea) bringing the total to 55 locations. In the standard VF printout, shown in figure (1) the total deviation display (1-E) shows the difference in dB (1 dB = 0.1 log unit) between the patient’s recorded sensitivity and the sensitivity of an age matched control group. This means that if a value of -10 is recorded in the total deviation display, it means that the sensitivity has decreased by -1 log unit from what it is predicted to be in a normal population. To make this comparable, we can say it is decreased by a factor of 10 compared to the sensitivity of the age matched control group, while -3 means it is decreased by -0.3 log unit or a factor of 2. The sensitivity differences are plotted in a probability map of the field called the total deviation plot (figure 1-H). The plot displays how much significant these deviations from the age matched normal population on a coded scale ranges between 5% and 0.5% [(39)]. The pattern deviation (figure 1-F) is a very important component of the printout; it reflects how much focal depressions found in the visual field, which is a good indicator of glaucomatous field defects. The pattern deviation probability plot (figure 1–I) measures the probability of these focal depressions to occur in a normal subject, and it is coded according to the probability symbols (figure 1-K) found in the printout. In addition to the 24-2 program, other test strategies used by the clinician include the 30-2 and 10-2 programs. The most widely used testing strategies in SAP protocols are the 30-2 and the 24-2 programs. The 30-2 program tests more points across the visual field, a total of 76 points out to an eccentricity of 30 degrees. In the case of the 24-2 program, the points are fewer and the locations are spaced 6 degrees apart. The 10-2 program, which is used for more advanced stages of glaucoma, covers 10 degrees of fixation and tests 68 locations spaced 2 degrees apart. The 2 degree spacing allows for greater spatial resolution in patients with more advanced visual field defects. Until recently, a full threshold algorithm was used to obtain HFA. The original design of this strategy was to perform a 4-2 dB double reversal staircase testing procedure, which usually takes between 10 and 20 min/eye to complete.

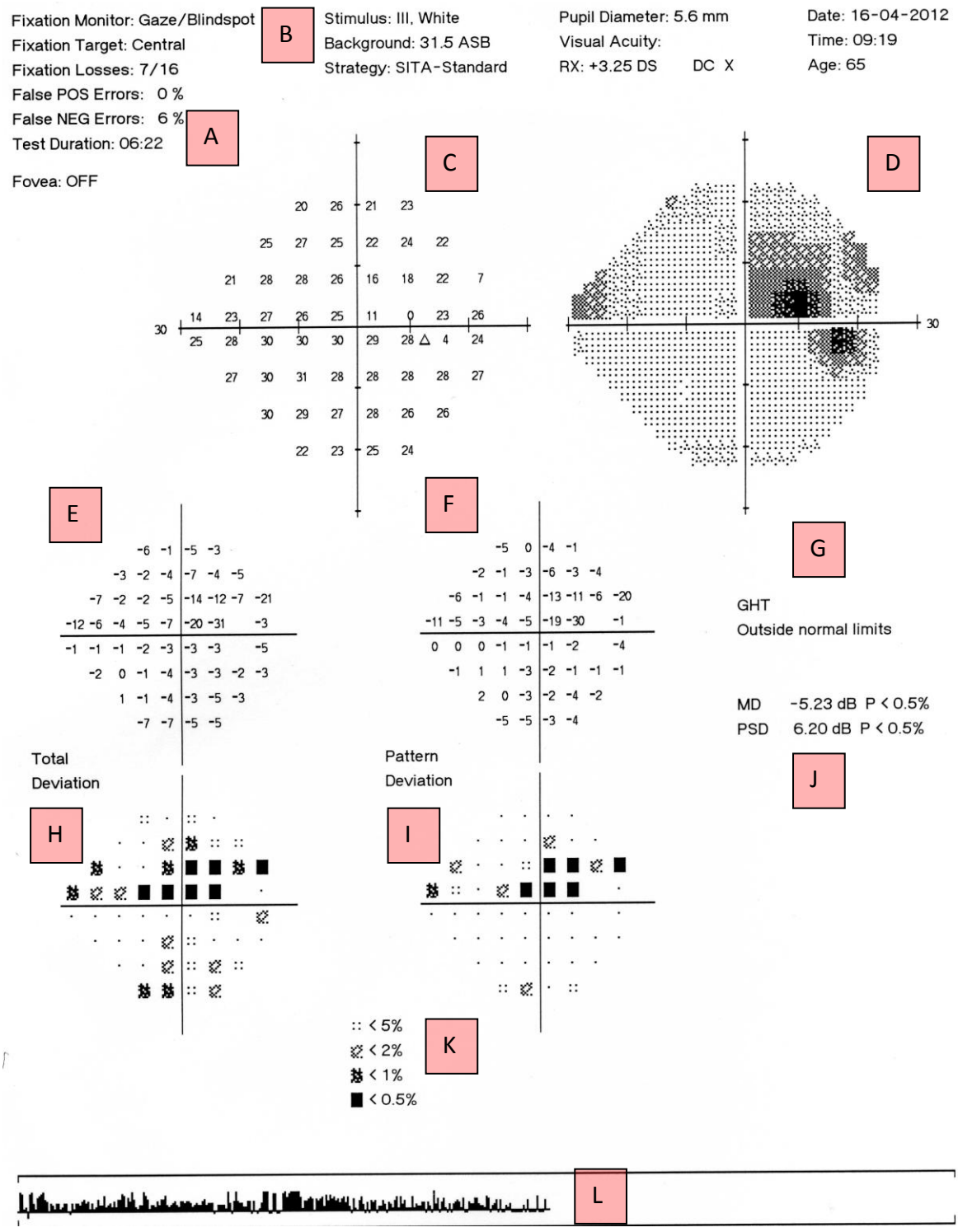



Figure (1) shows Basic components of the SITA standard 24-2 printout. Reliability parameters (A), stimulus parameters (B), threshold printout (C), graytone printout (D), total deviation (E), pattern deviation (F), glaucoma hemifield test prompt (G), total deviation probability plot (H), pattern deviation probability plot (I), global indices (J), probability symbols (K), and gaze tracking graph (L).

The lengthy testing time created more problems that directly influenced the test reliability such as the fatigue effect, leading to a need to reduce the duration of the test, and the SITA strategy was developed [14,40]. The SITA standard procedure was a breakthrough in terms of patient's convenience and reliability, as it approximately halves the testing time compared to the full threshold algorithm, and the SITA fast procedure results in an additional decrease in time [9,41,42,43].

### 1.2.3 Full threshold and SITA standard strategies

For decades the Humphrey field analyser offered the gold standard visual field test using the full threshold strategy, which showed many shortcomings listed earlier, but it remained the best available method for detection and monitoring of glaucomatous visual field losses. The introduction of SITA with its new way of algorithm calculation rectified some of the problems raised by the lengthy full threshold test and offered new level of better reliability. After its introduction to the Humphrey analyser and its integration to its standard software package, it became more popular and the majority of clinicians prefer to utilize it on a day-to-day practice. In reality, the SITA is not a true full threshold strategy; it is a computer generated program that was developed for the field analyser II (Humphrey systems) that reduced testing time. Through its progress and development, the SITA standard has shown to reduce the test duration by approximately 50%, while SITA fast reduced it by 70% [9,41,42]. This is accomplished by using different algorithms and newer probability techniques, including the use of information from the surrounding points and threshold values in the age-matched population; both normal controls and glaucoma from each location. There was a significant reduction in duration after elimination of retest trials for the 10 stimulus locations used for the calculation of short-term fluctuation in the full threshold algorithms, thus changing the method in which false positive and false negative indices are calculated, and using a protocol to maximize the likelihood procedure for estimating the retinal sensitivity threshold [14,40]. After the significant change in the way visual field testing was offered to glaucoma patients, it was suggested that SITA algorithms might replace the full threshold strategy, which has been the gold standard for detecting and monitoring of glaucomatous visual field defects for more than 25 years [9,14,41]. Budenz el al [44] evaluated the visual field defects in patients with glaucoma using the SITA standard and full threshold algorithms to determine whether results from these procedures can be compared when monitoring a glaucoma patient. They performed the two tests on 77 patients with glaucoma on two occasions for one month, and then the severity of defects were evaluated and graded on different scales. They concluded that glaucomatous defects were measured in their sample shallower using the SITA algorithms but were approximately the same size and severity compared with full threshold

measurements. They suggested that care should be taken when using threshold values to compare glaucomatous defects in a patient when converting from full threshold to SITA algorithms. Similar results were also reported [15,45] highlighting the difference in threshold values between the two tests. However, other studies such as Sharma et al [42] reported no significant difference between the two algorithms in their threshold values. Over years, assessment of glaucoma using SITA has gained widespread clinical usage.

### 1.2.4 Suprathreshold perimetry

Suprathreshold testing works in a different way. The stimuli are calculated to be above the expected normal threshold of retinal sensitivity. If the patient responds to these "above normal" points it is assumed that no significant visual field defects exist and most likely the subject does not experience visual field problem. Only when the response is missed, the suprathreshold test can highlight a pattern of visual field defect that needs to be investigated. This test is good for screening of normal and glaucoma suspect subjects, where less uncertainty and better retinal sensitivity are expected. Suprathreshold for its ease of use and yes or no rule may be easier tasks to perform with patients who are at risk for glaucoma and have little or no experience with perimetry. One of the major pitfalls of suprathreshold test is its low sensitivity to shallow and small visual field defects, which limited its use for screening and epidemiologic studies and not for monitoring or diagnosis of glaucoma [4,46,47]. Similar to the full threshold strategies, the suprathreshold testing shows high level of variability and low test-retest repeatability [48].

### 1.2.5 Glaucomatous visual field defects

Glaucoma is the most common cause of blindness in many countries. Primary open-angle glaucoma is the most common presentation, and it is a significant public health problem. It is estimated that 45 million people in the world have this type alone [6]. In addition, both open angle and angle closure glaucoma are the second leading cause of blindness worldwide, with approximately 8.4 million people being blind as a consequence of suffering glaucoma [6,7]. The main problem with glaucoma diagnosis is that it requires a clinical diagnosis to start the clinical management plan as early as possible, while there is no gold standard to measure the presence or progression of the disease [7]. Although visual field testing has been investigated and research into this area has produced different generations of technology and new testing modalities, there is no definite method of diagnosis of visual field defects that can reach the level of certainty. Most of the available tests have significant concerns with reliability and repeatability, which make these tests relatively the best available but surely not the best we are looking for. Assessment of the

visual field defects caused by glaucoma should be preceded by careful understanding and explanation of the damage caused by glaucoma and how it is progressing over time. Glaucoma is a chronic neurodegenerative disease characterized by loss of retinal ganglion cells, resulting in distinctive changes in the optic nerve head (ONH) and retinal nerve fibre layer (RNFL). It is defined as a progressive, chronic optic neuropathy in which intraocular pressure (IOP) and other currently unknown factors contribute to damage and in which, in the absence of other identifiable causes, there is a characteristic acquired atrophy of the optic nerve and loss of retinal ganglion cells and their axons [(7)]. Functionally, glaucoma causes loss of all three primary retinal ganglion cell subtypes, the parvocellular, magnocellular, and small-bistratified. Structurally, Glaucoma causes structural damage to the ONH, RNFL, ganglion cell layer, and inner plexiform layer [(8)]. Glaucomatous visual field defects are distinctive in how it starts and its progression pattern. Glaucomatous visual field defects usually have a distinctive pattern that follows the anatomical retinal nerve fibre layer anatomy, respecting the midline, and confined in its extension and progression to one hemifield. Functional deterioration probably becomes apparent from the visual field test after further neuronal loss has occurred, although visual field progression is often the only clinical sign that changes in the patient's vision have taken place. Perimetry methods are used to chart the course of disease in patients with glaucoma, where a progressive course will be assessed by the pattern of progressive visual field loss, indicating worsening of the disease. These visual field defects are summarized in figure (2). The purpose of testing the field of vision in a glaucoma patient is to detect and monitor the loss of ganglion cells, which has a specific anatomical configuration, mapping the arcuate fibres around the fovea centre, radiating from the optic disc but not crossing the horizontal midline as shown in figure (3). This unique anatomical configuration yields the characteristic shape and pattern of visual field loss in glaucoma. An early focal loss of ganglion cells will usually give rise to a focal paracentral scotoma, or nasal stepping, which typically extends into an arcuate scotoma. When multiple focal losses coalesce they form a full arcuate defect in one hemifield, most of the time earlier than the opposite hemifield so the glaucomatous visual field defects in the two hemifields are not matched equally in the size and depth. An advanced glaucomatous field loss may develop into an altitudinal defect, which involves almost the entire hemifield with a focal sparing of the central 5-8 degrees. Occasionally the glaucomatous visual field defect will affect the central (temporal) ganglion cells, which will give rise to an early central visual field defect. The glaucoma hemifield test is actually testing the likelihood of any focal defect in one hemifield matches its fellow similar part in the mirror image of the hemifield, and it will consider this focal depression or loss as outside normal limits when it is significantly lower in sensitivity than its similar part in the opposite hemifield.

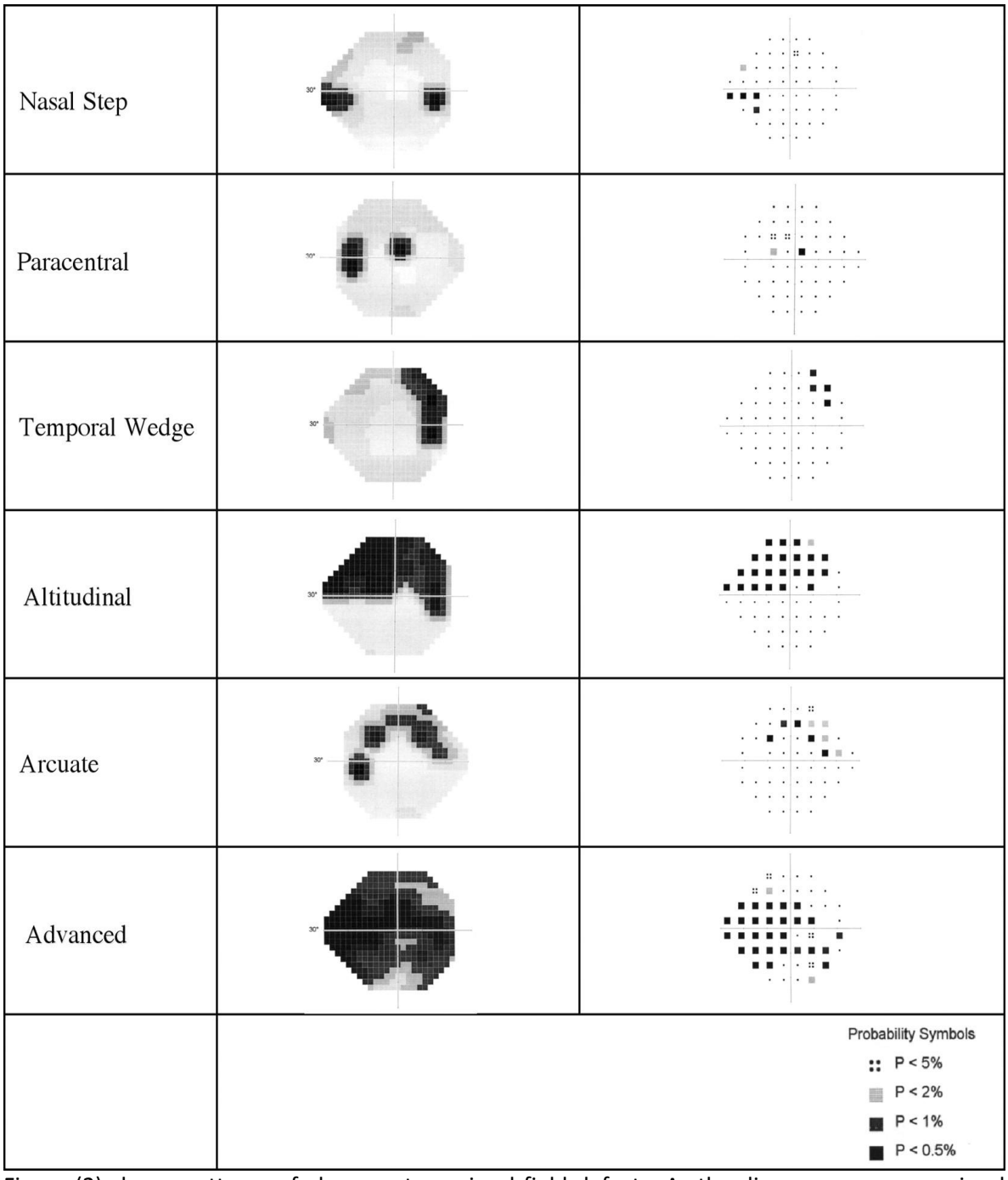



Figure (2) shows patterns of glaucomatous visual field defects. As the disease progresses, visual field defects become more apparent and isolated focal depressions start to coalesce forming a single arcuate defects, which is the classic appearance in moderate to severe glaucomatous visual field defects. The arcuate defects will progress encroaching the centre and finally affecting the central 5-10 degrees. Central visual field defects are not uncommon; it is a common presentation of normal tension glaucoma, where temporal ganglion cell fibres are affected earlier than other fibres.


Figure (3) demonstrates the anatomical distribution of the retinal nerve fibre layer in the two hemifield (right) and how the two hemifields represent the mirror-imaged distribution of the nerve axons in an arcuate configuration around the centre. Glaucomatous defects will usually start as a focal notch (loss of fibres) at the optic disc, which will be reflected in the visual field test as a focal field defect. Based on this unique anatomical configuration, the glaucoma hemifield test (left) is testing the probability of a focal depression in threshold sensitivity being due to glaucoma by comparing pre-set parts of the field of vision in one hemifield to its similar part in the opposite hemifield. The glaucoma hemifield test register as "outside normal limits" when the two mirror images are significantly different in sensitivity. *Heijl et al IOVS 2009;32:234-245*

## 1.2.6 Variables affecting reliability of SAP testing

Perimetric variability has multiple sources and it has been shown that it occurs on a neuronal level [(49)]. Most importantly it is the effect of focal scotomas on the adjacent normal ganglion cells. As the disease progresses, the edges of isolated scotomas and encroaching arcuate defects will fluctuate significantly and give rise to a high rate of variable responses. This problem will be worsened when fixation losses increase as the disease progresses, which is one reason why SAP protocols tend to give higher than normal variability as glaucomatous visual field defects progress [(11,12,16)]. Although action potentials of neuronal activities have consistent amplitudes, with ongoing disease process the amplitudes decrease and latency delay and become unpredictable [(50,51)]. Both short-term (intra-test) and long-term (inter-test) variability (fluctuation) were reported as major problems in standard perimetry, more prominent in patients with glaucomatous defects and decreased sensitivity, with the main effect coming from the physiological responses of affected ganglion cells and how the responses fluctuates with progression of the disease [(52-57)]. There are well known artefacts and false visual field defects that can be produced by a well calibrated standard HFA test. In order to distinguish these artefacts and false results from true defects, the sources of tests variability should be known and highlighted when a questionable test is produced.

### 1.2.6.1 Instrument variables

1- The background luminance of the perimeter bowl determines the level of retinal adaptation, which is essential factor in field testing and in defining the shape of the hill of vision [58].

2- The stimulus size is another factor in the testing procedure. In SAP testing, the size is usually constant throughout the test while the intensity is variable. Most automated perimeters use the Goldmann size III target (4 $mm^2$) as a standard [5].

3- The duration of target stimulation projected to the subject's eye can alter the response significantly; the longer the duration the better and easier it is seen, and the shorter duration will make the target harder to detect. This phenomenon is related to the physiological visual function of temporal summation [58-60]

4- Maintaining a steady fixation is crucial to the production of accurate visual fields. With the new SITA short duration strategies the fixation has improved [4,46].

### 1.2.6.2 Patient variables

1- Uncorrected refractive errors cause defocusing of the test target which make the target falsely unapparent and that is reflected on the retinal sensitivity.

2- Media opacities such as cataract and corneal haze, causes a well-known error in the visual field known as generalized depression in retinal sensitivity. Checking visual acuity prior to performing visual field test is essential to note any changes that can interfere with the patient's ability to observe target clearly.

3- Pupil diameter is another important factor, and it is routinely checked in most of the SAP tests nowadays. The smaller the pupil the less amount of light enters the eye and the field will artificially look more constricted, especially in diameters less than 2.5 mm which can give a pattern of generalized depression in retinal sensitivity [59].

4- There is a linear decrease in retinal sensitivity occurs with increasing age [61]. This decrease is more pronounced in the peripheral visual field than the central field points [62].

5- Ptosis and upper lid drooping are common causes of depression of the superior visual field; the more the obstruction of the upper lid the more points to be lost or counted as defective in the peripheral visual field.

6- Level of test-taking experience is another important factor that is specifically related to the SAP testing. There is almost always a clear learning curve need to be considered for SAP perimetry. The learning effect is greatest between the first and second tests [59,60].

7- Patient fatigue from prolonged testing procedure, such as full threshold strategies, may lead to decreased retinal sensitivity [63]. Fatigue is counted as the limiting factor when an attempt is made to increase accuracy by increasing test time [63,64].

8- Other important factors related to the subject's convenience, comfort, cooperation and comprehension of the test. The level of motivation sometimes plays a role in the quality of test-taking process. All these factors can affect the response of the patient either to selective test locations or generalized poor test-taking [3,63,64]. Visual attention can have significant effect on retinal sensitivity thresholds in standard perimetry [65].

9- Instructions given to the patient before the test can have a substantial effect on the subject's responses. It has been shown that substantial variations in the results of standard HFA perimetry occurred by instructing subjects in different ways, all are generally accepted [66].

## 1.3 ELECTROPHYSIOLOGICAL ASSESSMENT OF THE VISUAL FIELD

Electrophysiology has been tested extensively in visual pathway assessment over the past few decades. There have been several successful attempts to examine the relationship between specific electrophysiology test protocols and specific ganglion cell function loss such as the Pattern Electroretinogram (PERG) and Visual Evoked Potential (VEP) [67-69]. Looking at the ability to accurately detect glaucomatous visual field defects, comparing the standard achromatic static threshold (Humphrey - white on white) visual field testing to electrophysiological assessment, important differences can be observed, related to the factors affecting the final outcome and the level of variability and reliability [70]. In addition, for some patients it is very difficult or even impossible, to obtain reliable visual field measures due to various reasons related to the patients themselves or the requirements of the testing procedure [71]. Ganglion cells and optic nerve fibres (ONF) are the selective structures mostly affected in glaucoma, thus monitoring the anatomical and functional progression of glaucomatous defects mainly targeting these structures is of paramount importance.

### 1.3.1 The visual evoked potential (VEP)

The VEP test records the potential of the occipital lobe in response to pattern or flash stimulus projected to the retina. The visual stimulation which starts at the retina and ends at the visual cortex can reflect the integrity of the visual pathway. The technique is standardized by the International Society of Clinical Electrophysiology of Vision (ISCEV) [81]. One eye at a time is examined by scalp electrodes placed on the occipital scalp. The standard version of the test uses

single midline recording electrode (channel), which records the activity from both hemispheres. In this form of recording montage the test only examines the response from pre-chiasmal visual pathway. Other protocols use three channels recording; one on the midline position and one lateral to the midline on each side. The three channels recording measures the responses from the post-chiasmal pathways and compares the responses from the two hemispheres to each other [81,82]. The final output is an average of about 100 responses. This high number of responses is essential in order to confirm the intra-test repeatability of the responses. The pattern reversal is the standard form of testing and it is the most useful and reliable with a black and white checks reverses at a fixed rate 2 per second (2 Hz). Different protocols could be applied testing different functions of the visual pathway. The pattern onset and offset is another type of reversal that is used for assessment of visual acuity level [82]. Flash VEP is another type of stimulus that is usually used in infants, uncooperative patients, where media opacity is significant, and for testing the response of peripheral optic nerve fibres in addition to the macular bundle [70-72,81]. A patient should take the pattern reversal VEP test while undilated and optimally refracted to guarantee good image projection on the retina. Flash VEP can be taken with fully dilated pupils to maximize retinal illumination. Figure (4) shows the most important pattern reversal VEP test parameter that gives an indication of the integrity of the visual pathway is the latency of the positive wave at approximately 100 milliseconds, the P100 [81]. The latency varies among individuals according to their age and ethnic background, with an average range of 95-115 milliseconds [71,82]. Similarly, figure (5) shows the flash VEP test with a second large positive peak at approximately 100 milliseconds, which marked as P2 peak component. Pattern VEP is more reliable with less variability among subjects, while flash VEP is more variable and shows big variations in the waveform configurations and latencies among subjects, which makes its clinical value much less than the standard pattern VEP [70,81]. Despite its usefulness in diagnosing and monitoring of optic neuropathy, the benefit of pattern VEP is limited in the diagnosis of glaucomatous visual field defects, as it does not produce a topographical map of the visual field. Conventional VEP could be altered in the presence of ganglion cell dysfunction, mainly the P100 component, and it is considered an objective and reproducible measure of the function of visual pathway [72]. Although significant changes could be detected in glaucomatous visual field loss, VEP is not fully capable of identifying or assessing focal field losses, and is therefore of limited use in glaucoma. The reason for this limited use in the detection of focal field losses is that standard VEP does not have sufficient discriminatory power due to significant overlap of amplitude values between glaucoma patients and normal population [72]. VEP also does not provide a topographical measure of local defects, which is an important type of data presentation required for diagnosis of glaucoma and monitoring its progression. In addition, Standard Pattern Reversal VEP (PVEP) usually reflects

macular function as the first base in visual pathway, which is the structure that does not show significant changes in early or moderate glaucoma, and moreover, if it is damaged due to any other reason it will greatly influence the final results. Thus the P100 latency in glaucoma does not appear to be useful in clinical practice [72,73], although in many studies it was reported that abnormal P100 component detected in about half of glaucoma population [73-75]. Other research work was done on the use of VEP in glaucoma and significant delays in P100 latency was reported [76]. Parisi et al [70] compared normal controls to different types of glaucoma. P100 Latency was the main outcome measure in this study. They found that there was a significant delay in P100 latency (27.8 milliseconds) in all open angle glaucoma patients compared to normal subjects, with a sensitivity of 100%. Their results showed a marked level of sensitivity in identifying well-established glaucomatous defects, in patients with a known glaucoma. However, this study was not designed to randomly assign the test to identify early glaucoma changes among patients and normal subjects, which could have represented a more reliable approach. It also did not provide any information about the use of this parameter in the detection of early glaucoma changes or subtle defects. Conversely, of (Parisi et al) results; Grippo and colleagues [77] reported that contrary to previous reports, prolonged VEP delays were present in a minority of patients with glaucoma. They attributed these results to possible delay in VEP signal which is not a good standalone indicator of damaged, as opposed to dead, retinal ganglion cells. They also suggested another explanation that only few patients exhibited evidence of damaged ganglion cells. In conventional VEP the responses are summed from multiple striate cells in different orientations, which is also dependent on how healthy the macular function is. This summed response is also influenced by cortical representation of the stimulus size and location, which could give an overall delay or reduction in amplitude throughout the field, not adjusting for the amplitude and latency differences between peripheral and central locations. Latency of the P100 component was confirmed in some studies to be related to the degree of demyelination of neuronal cells, when disrupted in inflammatory condition, which is not the pathology involved in glaucoma. The latency itself could not be used as a valid parameter for detection of glaucomatous damage even if it yielded a statistically significant delay compared to normal. This delay in case of glaucoma is usually a mild, insignificant and limited change which is not correlated with the underlying glaucoma damage [73]. Research work continued in other studies trying to identify the most sensitive parameter in electrophysiological testing that could be measured with reasonable sensitivity and specificity, and which has a good predictive ability to detect early glaucoma defects. The Pattern Electroretinogram (PERG) was thoroughly investigated and was found to offer reproducible and significant data in its N95 component in glaucoma patients [78,79].

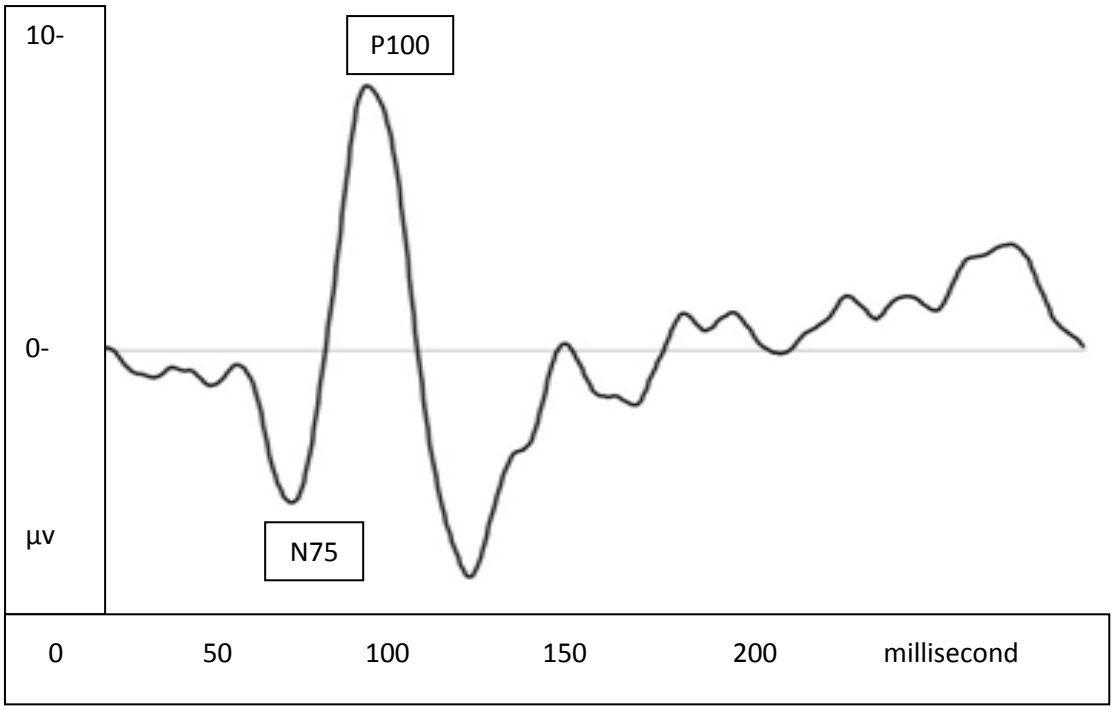



Figure (4) shows the classic waveform of the standard pattern visual evoked potential PVEP. The first negative peak is at 75 millisecond (N75) followed by a large positive peak at approximately 100 milliseconds (P100). The latency and amplitude value of the P100 are the most important components as diagnostic parameters.

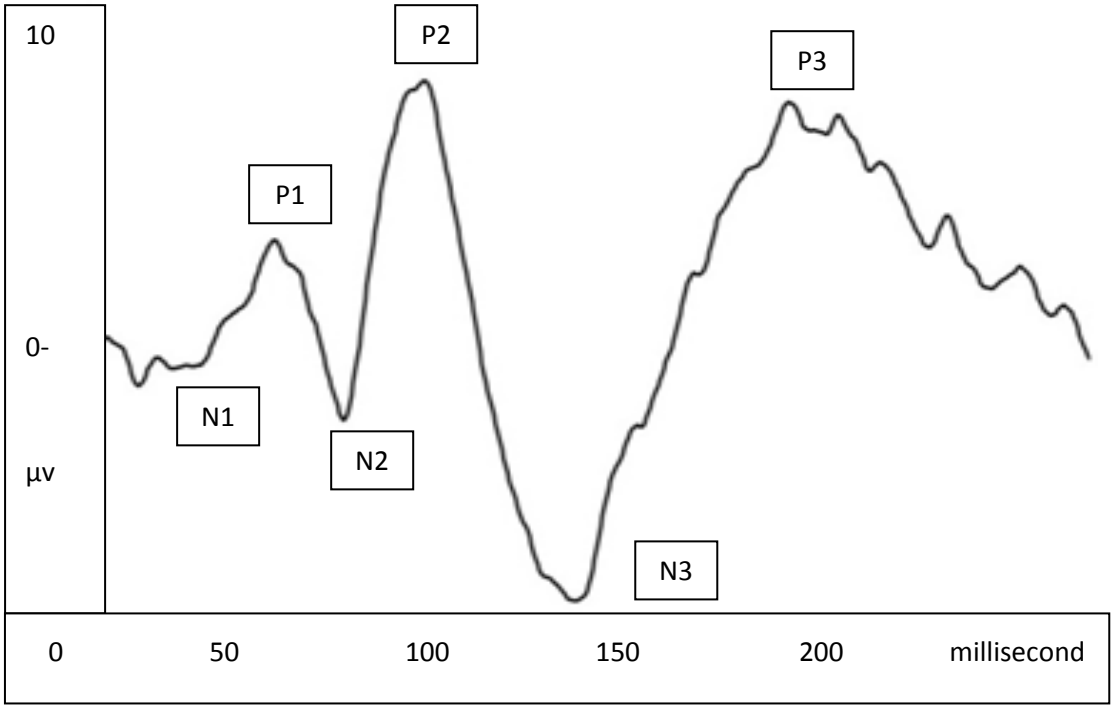



Figure (5) shows the classic waveform of the diffuse flash visual evoked potential FVEP. The first 2 negative peaks are marked as N1 and N2 approximately at 35 and 75 milliseconds respectively. The P2 component is the one bears the most important diagnostic value approximately at 100 milliseconds. *Author's copyright.*

In some published studies the PERG showed a level of sensitivity and specificity comparable to that of many other psychophysical tests used in detecting early glaucoma [78-80]. Although PERG is sensitive to glaucoma changes, it also processes these changes as an overall reduction in retinal sensitivity, mainly ganglion cell function, without giving information on focal mapping. It became obvious from clinical and research experiences that for any electrophysiological test protocol to be used in studying glaucoma it should obtain localized individual recordings within the field of vision, and should be able to present a topographic measure of various retinal sensitivities.

## 1.3.2 Multifocal electrophysiology testing

Multifocal electrophysiology testing (MET) techniques seem to offer some additional objective criteria for functional visual field assessment. MET is designed to test multiple locations of the entire retina simultaneously through projecting modulated patterns with preset contrast onto the retina, which gives a final topographic map of responses that could reflect the retinal function objectively. Extensive work has been undertaken over the past two decades in order to improve and stabilize testing protocols toward more sensitivity, less influence by background noise and better test taking conditions. There are two main types of multifocal testing available at the moment; we will discuss each of them in order to identify benefits and limitations of each technique, especially in the detection of glaucomatous visual field defects.

### 1.3.2.1 Multifocal Electro-Retinography (mfERG)

mfERG was developed by Sutter and Trans in 1992 [83]. It was designed to improve the concept of objective functional assessment of retinal diseases. Unlike Flash ERG, which stimulates the entire retina and measure its responses as a whole, presenting the response as a single waveforms, the mfERG provides individual stimuli to different locations and produce individual responses for each location simultaneously. The multifocal response map could be produced as a topographic colour coded presentation of all tested locations or as individual waveforms showing amplitude and latency

#### 1.3.2.1.1 Theory and technique

The recording technique used in mfERG is known as the pseudorandom m-sequence, which is the fast reversal of multiple hexagons pattern, usually contains 61, 103 or 241 hexagons. During stimulation the screen display seems to be flickering as each hexagon is being reversed in contrast pseudorandomly between black and white patterns. This sequence allows each hexagon a chance of 0.5 of being black or white at any given time [84]. While the subject views this display, a single

continuous ERG recording is obtained using the same electrodes and amplifiers employed for standard ERG recording. The 103 or 241 focal responses are generated and calculated, with each response derived from a particular hexagon, representing the entire visual field of 25-30 degrees on each side of central fixation [85]. The response components called kernels which are caused by non-linear dynamics of the responses [84]. Two waveforms are generated from the mfERG; the first order kernel, which is a biphasic waveform similar to the standard ERG waveform reflecting the retinal response to a focal flash, and it is arising from the outer retinal and photoreceptors layers of the retina, mainly the cones [86]. The second order kernel is smaller and has higher signal to noise ratio (SNR). It was initially proposed that this second order kernel arising from the ganglion cells, however recent work suggested that it is a measure of the retinal temporal interactions (Fast Adaptive Processes) and it is not a response generated by a specific retinal cells, it is rather a measure of how mfERG response is affected by preceding flash due to non-linear adaptive mechanisms of the retina [86-88].

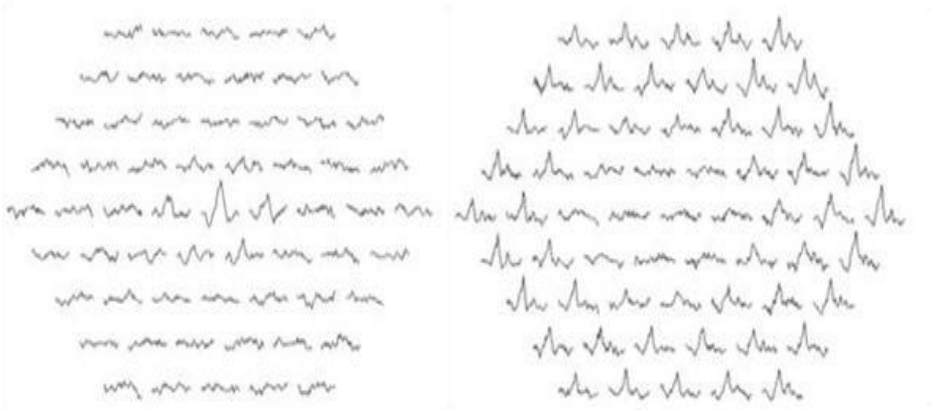


Figure (6) Two abnormal mfERG results; the left plot is from a patient with Retinitis Pigmentosa (RP), where peripheral responses are markedly reduced and central responses within normal, corresponding to the pattern of peripheral sensitivity loss associated with RP. The right plot is from a patient with Stargardt's disease (macular dystrophy), where there are markedly reduced central macular responses. *Author's copyright.*

### 1.3.2.1.2 mfERG and retinal functional assessment

Since the early introduction of mfERG it has shown a very high correlation with many retinal diseases. Many studies tested the ability of mfERG technique to detect photoreceptors dysfunction, macular dystrophy, and acquired retinal diseases such as diabetic retinopathy and drug toxicity. The results were promising, especially the analysis offered by the first order kernels in outer retinal and photoreceptor diseases such as Retinitis Pigmentosa, Stargardt's disease (figure 6), among other congenital photoreceptor diseases that could show locations of reduced sensitivity [89-92].

### 1.3.2.1.3 mfERG in Glaucoma

mfERG has a clear role in the diagnosis of focal retinal pathology by measuring its responses in predefined set of testing points. Its introduction to the assessment of glaucomatous damage was started by Sutter and Bearse in 1995 when they reported that ganglion cells contribute to the human multifocal ERG [93]. They provided some evidence to indicate the contribution to glaucomatous damage and its effect on mfERG. However, many subsequent studies have questioned the existence of human ganglion cell contribution to the mfERG, and the usefulness of mfERG in detection and monitoring of glaucoma was extensively studied [94,98-100]. Recently, many studies were conducted to measure the ability of mfERG to detect glaucomatous changes, and to provide a topographic sensitivity map that could be correlated with SAP results accurately [95-97]. In earlier studies there was a tendency to believe that second order kernels could reflect ganglion cell and inner retinal function, but there was no sufficient evidence to support this assumption [98-100]. In some other studies [101,102] they have found that the second order kernel is reduced in optic nerve atrophy and glaucoma and diabetic retinopathy, which damage the inner retinal layer. These studies provided the rationale for the use of kernel analysis in glaucoma detection and monitoring. Chan and Brown [103] in their work on the use of mfERG in ocular hypertension (OHT) found that both first order and second order kernels responses showed reductions in all OHT subjects. They explained these changes as a contribution from the inner retinal dysfunction associated with glaucoma changes [102,103]. They pointed out that the involvement of first order kernel changes could be interpreted as a significant change occurs in the outer retinal layers because of glaucoma [102-105]. These results could suggest that mfERG is useful in assessment of OHT patients, although reproducibility of these results was not clear and consequently how sensitive could it be for all OHT patients. Also the use of second order kernel analysis is limited by the high noise and small amplitude waveforms, which make the usefulness of its contribution as a solid and repeatable method for assessment limited, and it is not supported by many other studies [106,107]. Other studies have shown that mfERG is not a sensitive or reliable tool in the detection and monitoring of functional loss caused by glaucoma [106-108]. Fortune et al [97] found in his work no significant difference between mfERG responses from the affected and relatively unaffected hemifields, which could imply that there is a significant lack of spatial correspondence between mfERG and SAP finding. Other studies supported the contribution of ganglion cell and inner retina in the generation of mfERG responses by the loss of oscillatory potentials in glaucoma patients, which are generated mainly by the inner retinal cells [109,110,117]. Hood et al [112] has investigated in depth the contribution of ganglion cell and inner retina in the mfERG by performing pharmacological dissection of the retinal layers, using Tetrodotoxin (TTX) which blocks the sodium based action potentials. He found that ganglion cell contribution was detected in humans when contrast ratio of

stimulus was set to 50% instead of the standard 100%, which could again raise the question about optimum testing protocols and settings used in mfERG to selectively acquire ganglion cell contribution. Chan and Brown [103] in another study found that there is a difference in the level of mfERG responses between peripheral and macular locations. The macula showed a relatively larger reduction in response than the periphery in both waveform components of the second order kernel. He suggested as supported by previous work [113,114] that macular function in glaucoma is reduced to a significant degree, which illustrates the principle that central visual function provides important data for diagnosis of glaucoma. Chan explained his findings as the following: the second order kernel is derived from the inner retina, it may have similar characteristics to the PERG, where its negative peak at 95 milliseconds (N95) component arises from the inner retina, which is reduced in glaucoma. This may suggest that Müller cells and bipolar cells as well are involved in addition to ganglion cells [103,114]. In an attempt to improve the detection ability of mfERG in inner retinal dysfunction, novel mfERG stimulation techniques have been used in research work to isolate response components related to ganglion cell activity. In this regard, a number of new testing protocols have been developed to help in detection of inner retinal dysfunctions; such as Global Flash mfERG [109], Two Global Flash mfERG [115], Global Flash mfERG with Luminance Modulation [116] and Slow-Sequence mfERG [110]. A group of these mfERG novel protocols combine the standard protocol for multifocal stimulation with fixed and periodic global full field flash. These extra flashes are known to have the ability to extract a larger ganglion cell contribution [115-118]. Overall, the standard mfERG in its current testing protocol cannot provide accurate glaucomatous visual field defect mapping due to the lack of ganglion cell contribution in the formation of standard waveform.

### 1.3.2.2 The Multifocal Visual Evoked Potential (mfVEP)

As discussed earlier, the visual evoked potential (VEP) is an easily recorded procedure using standard scalp electrodes to record visual cortex activity in response to visual stimulation, and it provides an objective and reproducible measure of the function of the visual pathways from the retina and up to the optic chiasm in the single channel recording, and up to and including the visual cortex for multichannel recording [39,120-122]. However, the standard protocol for VEP is of limited value in the field of glaucoma diagnosis, mainly due to the lack of topographical representation of the visual field, and also because of the single averaged waveform output that is produced as an outcome of the entire visual field stimulation. Although VEP recording is very valuable in assessing the visual pathway objectively, in case of glaucoma study, multiple VEP stimuli and responses need to be implemented throughout the entire visual field to check for any focal defects accurately. This principle is valid theoretically, but it would be too time consuming in

clinical practice. This multi-VEP stimuli concept was first introduced technically in a new method by Baseler et al [162], which was based upon the standard multifocal technology [123], which handled the multifocal theory properly and effectively. The new method uses multifocal stimulation covering a central range of 56-60 degrees, using 58-60 multifocal pattern isolated stimuli. This type of stimulation can produce multiple responses and it can be recorded as individually spaced and focal responses of the entire visual field. The limitation of conventional VEP in the assessment of individual locations in the retina raised the idea of using the same principle of objectivity in obtaining information from multiple locations simultaneously rather than summed overall responses. The principle is to stimulate the retina on an isolated rather than cumulative basis, and to process these signals individually in order to draw a map of different retinal sensitivities on various locations. Successful recordings should detect in this case any small scotomas or subtle depressions that reflect early functional loss. The Multifocal Visual Evoked Potential (mfVEP) provided to a great extent this model of multiple simultaneous stimulation of the entire field of vision, presenting its results as a topographic map of the field of vision.

#### 1.3.2.2.1 Origin of the mfVEP signal

The first impression about the multifocal VEP is that it looks like multiple small VEP's fragmented in multiple points across the visual field. This is not true, as the mfVEP signal is different in its size, shape and origin from the standard VEP signal. Researchers have provided two main evidences that the mfVEP signal arises from visual cortex V1; firstly that there is a significant and repeatable polarity reversal as the signal moves across the horizontal midline, which means that the calcarine fissure is at least involved in the origin of this signal [124,125]. It is quite known that the cells in the calcarine fissure generate its responses reversed in polarity according to superior and inferior field stimulation. Secondly, a dipole study of the cellular responses to VEP stimulation showed in the analysis, using multiple needle electrodes, that the visual cortex V1 is the source of the mfVEP signal, and this was presented in many research studies [126-129]. There is evidence that the mfVEP signal has an extrastriate contribution; most likely due to the variability of the mfVEP signal shape and size across the field in response to similar stimulation, which means that another source must be contributing. It was concluded that the source of the mfVEP was primarily V1, but these additional dipoles could represent an extrastriate signal or a second signal generated within V1 but with a different type of orientation make it distinctly different from the primary signal [121,125,126]. This can be considered an evidence that pattern stimulation in general, such as PVEP and mfVEP, are similar in their origin, which has two different types of cells contributing in the formation of its signal, both striate and extrastriate shows that it is likely that the mfVEP, like the PVEP, has both striate and extrastriate contributions. However, the extrastriate contributions are probably

smaller in the case of the mfVEP [39,124]. Another important difference between conventional PVEP and mfVEP is that the latter has an independent response from each segment, without any averaging taking place in the final output. The segment stimulus itself goes through a random sequence of frame change every 13.3 milliseconds (ms). It can also reverse its contrast or stay the same according to a preset sequence called the m-sequence. On the other hand, the traditional pattern reversal VEP is reversed 2 times per second and the VEP response is obtained by significant averaging process and the records time-locked to the stimulus reversals [81,129]. Finally, the mfVEP response from each segment is not a true response that can be compared to the VEP signal, which is the case in all multifocal stimulation, where the output waveform is a mathematical extraction abstracted from the sequence of segment responses by a process called the cross correlation, which is the result of a correlation between the reversal sequence of each sector and the continuous record. This means that the mfVEP signal we see in each segment cannot be considered a true a – b wave component, but rather a mathematically driven signal from each segment. The details of this mathematical method are discussed elsewhere [84].

#### 1.3.2.2.2 Spatial resolution of mfVEP signal

Assessment of the signal quality; the noise portion and the true signal parts, is always dependent on the amplitude of each part of the signal. If the response is larger than the noise level then the quality of the signal is better, hence the spatial resolution will be better than the size of the stimulated region. Conversely, if the response amplitude is smaller and close to the noise level, then the resolution will be poorer than the size of the stimulated region. This signal analysis will become more evident in the peripheral locations, where the response is usually smaller as it has been shown that the response per unit area decreases with eccentricity, which will make the signal spatial resolution decrease with eccentricity [130,131]. However, this spatial resolution differences will not be the same in all individuals due to the inter-subject variability of the signal, and the variability of signal amplitude across the visual field between subjects. To precisely determine the spatial resolution requires a signal-to-noise (SNR) analysis. For this reason, a monocular mfVEP test need to overcome the variability in spatial resolution across the field in order to validate its results, thus a signal to noise analysis is needed to make these values useful. It is important to emphasize that when we are looking at any signal we have to think of it as two separate parts; a signal window and a noise window. The signal portion is usually identified by the expected latency of the signal, knowing that it should be within a specific range of time after the stimulus. This signal window contains both signal and noise inputs mixed together. However the fact that the ‘‘noise window’’ contains essentially no signal, can help in the calculation of the SNR values for all segments. [39,131,132]. It is also important to understand that the local amplitude mean is crucially

dependent upon the size of the signals, and its standard deviation (SD) will depend upon the consistency in size across individuals. The outcome of many studies confirmed the variability of signals across the visual field, which makes it easy to expect a significant difference between central and peripheral locations without being confused about the nature of these smaller peripheral responses if it is pathological or just normal variation. A big concern when dealing with visual field variability in a monocular test is the false negative rate, where classification of focal depressions as normal variation in the presence of an underlying defect, and false positive rate, where signal errors give rise to normal waveform response falsely. A careful SNR analysis of the visual field is required in monocular testing to obtain an accurate estimate of false negative rates. An interesting study calculated the false negative rate by field location for the noise window of Hood [39,132]. He studied 30 normal subjects using mfVEP technique and calculated a false negative error varied from 0% to 53%. He reported that the 6 lowest SNR cut-offs, 34% of the responses with no signal present were classified as ‘‘normal’’, while for the regions with the 6 highest cut-offs this value approached zero. This analysis is unique and it provided a quantified method to calculate one of the major errors in mfVEP monocular test. However, most of the commercially available mfVEP equipment does not have such analysis integrated within the testing programs, which force operators to run these variability checks manually and independently. Almost all studies undertaken to assess the variability of mfVEP test confirmed the need of a monocular test to have a method for overcoming the SNR variability between individuals, and that the 95% confidence interval levels at some locations in the field approach the level of pure noise, which makes it difficult for glaucoma assessment strategy, as glaucomatous defects will have to reduce the size of the signal to below the noise level for the monocular test to detect damage in some locations [39,71,132-135]. Variability in normal subjects showed a mean SNR varied by a factor of about 3, from about 2.0–6.0 in many studies [132-136]. The relatively small SNRs in a few individuals can be traced to high noise levels. Most of these variations were attributed to a variation in the size of the signals. It is this range of SNR values that creates a problem for tests of significance, especially those based upon analyses of monocular mfVEP test. Variability issues even among normal individuals pushed researchers into one of two directions to overcome this problem and make their data valid. One method is to standardize the results by comparing it to population normative data, which is difficult to establish due to big variations among individuals. The other option is to standardize the results by comparing it to normal responses closely matching the tested eye, such as the inter-ocular analysis, where one eye is compared to the other. One factor that makes the monocular test a standalone viable test, is its low tendency to cluster when significant points for normal subjects are tested, while in glaucomatous visual field defects the affected regions are

more likely to cluster because most of the close by points are affected by glaucomatous damage [136].

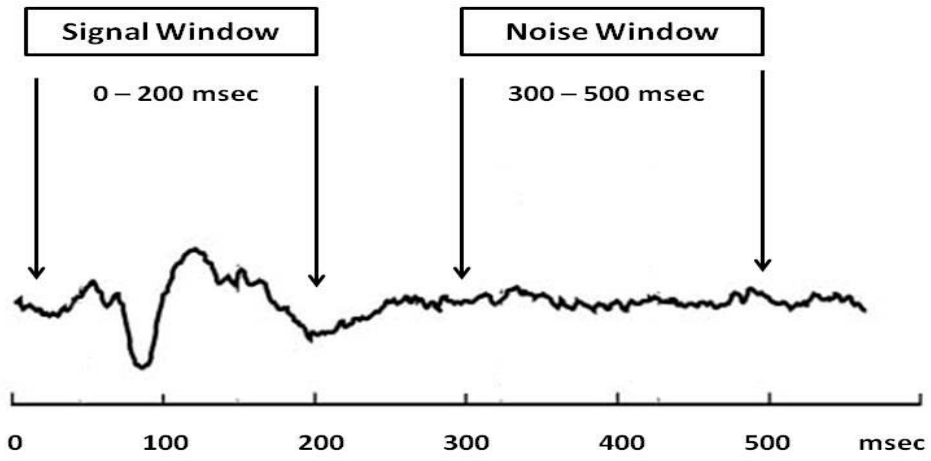
### 1.3.2.2.3 mfVEP and Bilateral damage; the monocular test interpretation

When inter-ocular comparison is the method used to overcome mfVEP monocular variability, other factors should be considered. The obvious shortcoming of an inter-ocular comparison for the mfVEP test is the possibility that bilateral damage will not be detected [136-139]. Defects in the same part of the visual field in both eyes (such as infero-temporal in one eye and infero-nasal in the other eye) will not be detected if simultaneously compared. Because of the inter-subject variability, it has been suggested by some authors [120] that a monocular mfVEP test is not viable. However it was reported [134] that notable success could be accomplished with a monocular test for glaucomatous damage using different analysis protocols or spatial resolution scales. They concluded that multifocal objective perimetry can successfully assess the visual field and be able to detect glaucomatous visual field defects. Furthermore, it may show a higher potential for identifying defects earlier than SAP perimetry. Another study [138] used the overall EEG level to normalize the results, which showed good standardization and validity. The whole concept of overcoming shortcoming of a monocular test is to use an effective signal-to-noise analysis. Doing so will enable the researcher to identify locations of true signals and differentiate between locations of low responses due to higher noise levels and those due to a true focal defect, whilst quantifying the response from different parts of the tested visual field in a measurable and standardized way [139]. The problem of deciding what constitutes a local defect is not unique to the mfVEP, as most of SAP tests suggest a focal defect at a probability scale, but it still has the same problem. The standard 24-2 test in SAP can easily define a cluster of points as abnormal if they collectively meet some criterion [140], but cannot tell if these focal defects are due to glaucoma or not. For example, 3 or more adjacent points exceeding the 5% level or 2 or more adjacent points exceeding the 2%, or 1%, level have been used as criteria [141]. The SAP analysis protocol typically uses some criteria to judge a focal cluster, such as all points have to be within a hemifield, because the cluster cannot cross the horizontal midline. This type of shortcoming of mfVEP is not easily detected as it is in SAP, because there is no designed software to check for false negative errors and intra-test variability in any of the commercially available mfVEP software. Some researchers provided some solutions for excessive false negatives by creating mfVEP monocular plots. Goldberg et al [134] suggested a similar cluster approach. They created a probability plot for monocular mfVEP test, and labelled the mfVEP probability plot as abnormal if 3 adjacent points exceeded 5% with at least one of the points exceeding 2%. However, the SNR method should be simpler and easier to practice in a monocular test, as the criteria is different, and perhaps more

reliable. There are two major differences which make the use of SNR more reliable; firstly, it includes all points and segments within the tested field without exclusion to peripheral rings. And secondly, the clusters do not cross the midline based upon the same logic used for the analysis of HFA. The continuous research work on mfVEP reliability was driven by a common belief that when it comes to test sensitivity, it has been suggested that mfVEP may be more sensitive in detecting glaucomatous damage than the HFA [134,142]. In order to utilize this superior sensitivity properly, different analysis protocols using the SNR values have been suggested which use monocular test data with reasonable reliability. Further analysis of the monocular test was carried out to identify the visual field defect depth in a mfVEP test. However, to determine the conditions under which either test may be more sensitive to glaucomatous damage requires a better theoretical understanding of the relationship between the amplitude of the mfVEP and the depth of the HFA defect. Hood et al [126] studied patients with unilateral visual field damage in an attempt to highlight a possible relationship between an existing sensitivity loss in SAP test and amplitude changes in mfVEP, trying to establish a practical definition of defect depth in mfVEP setup. He successfully developed a technique for calculating the HFA values for the tested regions of the visual field corresponding to each sector of the mfVEP monocular test display. He was able to create interpolated maps of both tests; using the total deviation scores of HFA and SNR values of mfVEP. In addition, he extracted some qualitative information about how these interpolated field maps can show where the mfVEP array of responses should meet with HFA losses. His method was promising, however, it remains an attempt to correlate the two different tests probability plots directly, which is a point of debate about its validity and accuracy, since each test has a different spatial resolution and probability scoring system. In his results he showed that the presence of good SNR value almost always indicates that the visual field in this specific region is good as well. But conversely, a small mfVEP response does not necessarily mean that there will be a visual field defect or that there is a confirmed glaucomatous damage in this location. A large response means that the visual field sensitivity should be reasonably good, and If it is not, the SAP visual field should be questioned because a repeatable and confirmed mfVEP response is more sensitive than SAP test results [39]. Monocular field analysis measures focal defects precisely, but the comparison between the two tests is essential in order to confirm or raise the suspicion about a focal defect in case of agreement or disagreement respectively. What makes these comparisons more sensible is that it comes from the same regions as focal responses (not necessarily scaled similarly), and the two measures possess comparable ratio scales that can be looked at independently but also collectively.

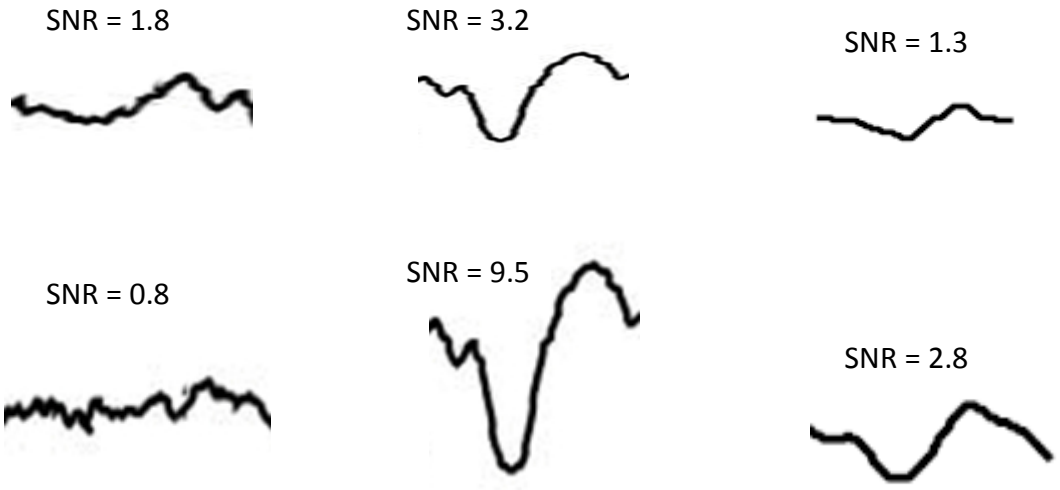
### 1.3.2.2.4 The need for Signal to Noise Ratio (SNR)

The mfVEP records, like all electrophysiological recordings, contain the signal of interest embedded in background noise. (Figure 7) illustrates how the relative amplitudes of the signal and the noise can influence the amplitude of the recording.




(Figure 7) The typical mfVEP signal is divided into two parts (windows) according to implicit time. The signal window between 0-200 milliseconds, and the noise window between 300-500 msec. SNR is calculated based on this division of the waveform. *Author's copyright.*

It is well known that the signal should be present within the first 200 milliseconds portion of the signal/noise waveform. The first part is called the signal and noise part, and the remaining part (200-500 milliseconds) will contain only noise. The idea of obtaining the SNR value is to compare the signal part, which contains the signal mixed with noise, to the pure noise part comes after the signal. If the signal is well formed and recording is good, then theoretically if we compare the signal window to the noise window we should get a ratio greater than 1.0, which means that the signal could be detected and distinguished from the noise contamination. The root mean square (RMS) is a mathematical method used to extract variable amplitudes, which does not depend on a particular aspect of the response waveform, as does the peak-to-trough measure (pure amplitude measurement), which was confirmed by many studies [126,132,142,146]. In other literature [39,71,133,134,143] different time windows were used, mostly between 0-165 milliseconds for the signal and 325-450 milliseconds for the noise. The benefit of performing SNR values in mfVEP monocular test is that noise is a very important factor that can influence the quality and the values of signal amplitudes, so it needs to be taken into account in order to avoid significant false negative and positive errors.




(Figure 8) Examples of SNR values showing the shape of the response. *Author's copyright.*

Inter-subject variability of signal and noise levels can create problems in the interpretation of test results; most importantly the noise can mimic a genuine signal where no response should exist. Various approaches have been implemented to solve this problem and easily differentiate the signal from noise using SNR ratio for each segment [39,131,132]. To obtain an SNR, the amplitude calculated using the RMS method of the signal window, which contains both signal and noise, is divided by an estimate of the RMS of the noise window. The RMS signal window is calculated individually for each segment, but the noise window is the mean average noise from all 58 segments multiplied by 4 channels. Using the average noise from all segments is a better method to standardize the noise value in the entire field as shown in previous studies [39,71,132,133]. Thus the SNR of an individual record is defined as the RMS of the signal window divided by the mean RMS of the 58 noise windows [39]. It is important to highlight that the SNR is not a measure of signal divided by noise from each segment. Rather, the SNR is a measure of the signal window, which contains both signal mixed with noise of any given recording, divided by a measure of the average noise level from all segments. So, if there is no signal present, then the SNR would equal 1.0. But practically speaking, under these conditions individual SNRs could be greater or less than 1.0 depending upon the noise in the individual recording. Figure (8) shows sample responses from control subjects chosen to show a range of SNR values. For those recordings which contain SNR values around 1.0, it is difficult to distinguish any true signal from pure noise. The larger the SNR value the better the true signal and the better the response from this specific segment, indicating a normal response. In figure 6, other waveforms with SNR values ranging between 2.8 and 9.5 show well-formed components, and reflecting a good to excellent response. With further

increases in SNR, the signals become larger, the waveforms more consistent and the examiner can confirm that the response is a true signal arising from this specific location [39,132].

### 1.3.2.2.5 Single versus multiple channel recording

The concept of single channel recording is based on using single midline recording electrode on the occipital bony landmark, called the bipolar recording, which records the responses through a single recording route and one reference point (figure 9). This method is currently obsolete for many reasons; most importantly due to the high variability and differences in the correlation between the visual cortex V1 and the electrode position in the midline. Several attempts have been implemented to achieve more than one recording in at least two different directions; horizontal and vertical to maximize the chance of improving the signal. A variety of midline placements have been used. There have been several attempts to improve electrode placement and the recording montage so that it can provide a representative and reliable signal. There is substantial variation amongst individuals in the location of the inion with respect to the calcarine fissure. One point is essential to be covered in all electrode montages, which is at 4 cm above the inion, ensuring that it is above the calcarine fissure in nearly all individuals [39,144]. Multiple channel (multichannel) recording was started by Klistorner and Graham [142] where they used 4 channels recording montage producing transverse, horizontal and oblique recording orientations. They found that electrodes placed lateral to the inion often improved the signal in these regions in a way that makes the recording more reliable and consistent. Following a 4 channel recording, 6 channels were used by Hood et al [126] and showed more confirmatory evidences that multiple channel recording can offer more accurate responses and less variability. In the analysis of these waveforms, the optimal channel is the channel at each location that produces the largest SNR of all channel responses (4 or 6 channels). Since its introduction, the multichannel recordings has become an essential montage in all mfVEP testing, and results of these tests have improved and showed significant less variability.


(Figure 9) Typical electrode montage for VEP recording; the active electrode could be a single channel placed in the midline 1.0 cm above the Inion, or multiple channels in the dipole position around the Inion. *(With permission; ISCEV standards publications for VEP testing)*

### 1.3.2.2.6 Variations in the normal mfVEP

#### 1.3.2.2.6.1 Variations between eyes

It has been documented that corresponding points in the visual fields for any image coming from the two eyes project to the same region of the brain [166-168]. For this reason, all anatomical variations in folding and orientation of the brain in relation to external bony and scalp landmarks are identical, which will make no difference in the placement of electrodes on recording from both eyes. This can be translated in terms of mfVEP testing as a monocular test that can be compared easily and accurately to the fellow eye as both field representations are identical [126,139]. However, it worth mentioning that minor differences do exist between the two eyes; noticeable amplitude asymmetry across the horizontal midline between the two eyes, a small inter-ocular latency ranges between 4-5 milliseconds also noted. This latency is related to the relatively longer time taken for peripheral fibres to transmit the signal as opposed to central parts, where the left eye leading in the left visual field and the right eye leading in the right visual field [39].

#### 1.3.2.2.6.2 Variations between individuals

Inter-subject variability is a major limiting factor for the use of mfVEP test in a larger population [120]. This variability has been shown repeatedly in standard central field VEPs and has been attributed to many factors; such as age and sex [137,143,147-150] cortical convolution and orientation of the calcarine fissure in relation to external landmarks (inion) [120,151-153], the eccentricity of the visual field tested points and degree of visual angle [154], individual parameters such as the conductivity of underlying tissues; such as scalp fat and skin thickness, bone and blood circulation, and perhaps equally important the general level of brain activity [155,156], and finally the testing setup and stimulus protocol conditions [157]. There is a wide variation in the location of the calcarine fissure in relation to the external landmarks [135,158,163-165]. The relative amplitudes of the superior versus inferior field responses are roughly correlated with the position of the calcarine fissure. Some factors such as cortical folding and orientation are very difficult to change or adjust. Other factors can be compensated or adjusted for, such age or scalp structures. Some other factors can be controlled to produce best quality signals such as stimulus and testing setup [138]. Referencing the tested eye and comparing its values to some standard values still the main concern for many researchers. Many attempts have been implemented which use the fellow eye in an inter-ocular asymmetry analysis to validate and make use of the signal values acquired from the tested eye, which showed good and compatible results [19,139-142,161,162]. The major factor that influences the quality of mfVEP recording is the cortical folding orientation to external landmarks, and even when inter-ocular analysis is used the problem still exists because both eyes are affected equally by how close and accurately the electrodes are placed relative to the visual cortex. However, this symmetry in influence has put a valid analysis protocol into practice, as minor visual field changes in one eye can easily be differentiated if they are compared under the same conditions as the fellow eye, without the need for reference to external normal values, assuming that one eye is normal or a valid reference for the other, such as in cases of unilateral optic neuritis or early unilateral glaucoma. If bilateral damage exists, which is the case in many diseases affect the visual field, this analysis protocol cannot be considered valid [138]. The shortcoming of the inter-ocular analysis pushed researchers back towards establishing a normative database for mfVEP test, which was faced again with the strong inter-subject variability issues. The variability of mfVEP among normal individuals made it necessary for researchers to standardize a suitable normative database that could be used for patients with an accepted range of standard deviation from the mean values for central and peripheral responses of the visual field. However, this normative data cannot be standardized as a universal database in any testing equipment, but many commercially available software packages use some form of normative database in their testing procedure to produce grading scale with probability maps; VERIS™ and AccuMap® systems

are examples of these software. Some authors [19,39,159,160] have suggested a more practical and suitable method for normative database standardization, which is for each clinic to create its own age-related normative data. Much care should be employed when using a standardized form of normative database for mfVEP; as the level of noise directly affects the ability of the test to detect early changes in the visual field in a number of ways, for example many signals could be either missed, or counted as false positive or false negative. However, mfVEP has shown good repeatability in many studies, even better than SAP protocols [142,146,161,162]. Individuals differ in the way the V1 region is folded, and this important anatomical factor will influence the amplitude of the mfVEP and quality of the signal [161]. This variation was evident in many studies; one of them [166] has shown that there is a wide variation among individuals especially in the central 21 degrees and how it is represented on the occipital pole. This means that at least the cells in the central 21 degrees can have variable orientations with respect to the recording electrodes, which can be noted in different individuals.

#### 1.3.2.2.6.3 Variations across the visual field

The mfVEP waveform and amplitude varies across the visual field in several ways.

1- As noted in many research studies [120,144,146,163], the responses from the superior and inferior visual fields are reversed in polarity which can be noted in the majority of subjects. This reversal originates from the orientation of the cells in the calcarine fissure; superior and inferior banks, which represent the superior and inferior visual fields. Because V1 is the origin of the mfVEP signal, which lies within the calcarine fissure, it is influenced by the orientation of its cells, which are lying opposite across the horizontal midline [39,144].

2- The responses vary in amplitude even from regions at the same eccentricity. Again the location of V1 relative to external electrode placement is crucial in the signal variability. However, cortical magnification is another important factor in the variability of signal within the same visual field. Peripheral signals are smaller, noisy and more irregular than central signals. That is why multichannel recording is considered in recent mfVEP testing protocols, to maximize the signal detection process at its highest representation. It has been shown that the signals are smaller just below the horizontal meridian than just above it [39,131]. Aine et al [167] provided one possible explanation. They recorded magnetically evoked potentials to focal stimuli in normal subjects. They were able to localize the various sources of the responses with dipole modelling. They found that occasionally if the field just below the horizontal meridian projects to the lower bank of the calcarine cortex, which is the case in many individuals, then the mfVEP signals from the region below the horizontal meridian, would be in the fold of the calcarine. This orientation will give the

cells a more perpendicular location relative to the recording electrodes making the signal weaker and smaller just above the midline.

3- It also has been shown that in some normal subjects there is a noticeable difference in the waveform configuration across the vertical midline [146,138]. For some authors this was considered evidence that there is an extrastriate contribution for the origin of the mfVEP signal [39]. The explanation given for this asymmetrical waveform shape is that if it is arising from the same location it should have been different in amplitude and latency but not in the shape of the waveform itself. This second source may be in the extrastriate cortex or in V1, but oriented perpendicular to the first source [39,146]. This naso-temporal difference in signal shape was attributed to the presence of blind spot in some literature [131,161], but looking at the difference carefully it is easy to discover that it occurs for regions far from the blind spot nasally, which does not support this explanation. Brenton et al [168] confirmed this small naso-temporal difference, which may reflect the small naso-temporal difference in sensitivity approximately 1.0 dB or less that has been reported for visual field measurements [39,161].

4- Another small latency difference can be seen along the midline between eyes in normal subjects. The left binocular visual field, which led by the left eye, is faster than the right eye left visual field with about 5 milliseconds, and the reverse is also true. The explanation offered for this latency is in the time it takes signals to arrive at V1 from the nasal as opposed to temporal retina [126]. Most likely, this small difference is due to the conduction time of the unmyelinated ganglion cell axons on the retinal surface. The signal is processed faster in the nasal retina, reaching the optic disc, than the temporal retinal ganglion cells [39,126,169].

### 1.3.2.2.7 mfVEP as an objective tool for visual field assessment

mfVEP is considered an objective method of visual field assessment as it does not require any interactive response from the patient to produce a topographic assessment of the visual field. Hood et al [39] have recorded mfVEPs from about 500 patients. In an attempt to draw a mfVEP signal profile for these subjects, approximately 300 were patients with glaucoma or glaucoma suspects and the rest were normal. Their findings were very important in outlining the potential clinical uses of the mfVEP as an objective tool in the assessment of glaucomatous visual field defects. Their experiences can be summarized in the following points:

1- Unreliability of SAP tests has been always a source of confusion because it influences the management plan and occasionally cannot guide the clinician to make a decision. It is well known that many patients do not perform well on a SAP test for variable reasons. This

category of patients is more suitable for mfVEP test as it does not require much interaction from the patient and it is not dependent upon their responses, where they usually find that reliable mfVEP records can be obtained from patients with unreliable visual fields. Occasionally, individuals who yield poor visual fields, will produce poor mfVEP test results as well. Most of these subjects have fixation problems or have issues with proper cooperation or comprehension to take the test.

2- Occasionally the patient will be able to take the test reliably, but the results are either questionable or inconsistent visual fields with the clinical picture, even though the reliability indices are within the normal ranges. Those patients can benefit from mfVEP test as an additional complimentary test, where it can confirm or decline the results
3- The mfVEP test can also add more confidence and confirmation to the visual field results, especially when focal visual field defect is present. Clinicians in these situations would like additional evidence because a decision regarding clinical management may depend upon it.
4- The use of mfVEP in the detection of early damage and progression has been well documented, especially in addition to the SAP visual field assessment, where the standard visual field test is less than optimal in these cases

#### 1.3.2.2.8 Clinical applications of mfVEP

mfVEP can work as a conventional VEP in detecting optic neuropathies of many types. Because this procedure records the cortical activity in response to visual stimulus, it can test the integrity of the visual pathway by defining local defects in the field of vision. There is much research work done on the role of mfVEP in certain clinical cases that showed good results other than the detection of glaucomatous visual field defects.

##### 1.3.2.2.8.1 Early detection of glaucoma

Based on their findings, Hood et al [39] and Chen et al [145] tried to answer the questions; can the mfVEP detect damage earlier than the HFA? Will the mfVEP be useful in tracking progression? They concluded that although these questions are important, they are currently unresolved. However, the ability of the mfVEP to detect progression will be limited by its repeatability [39,145]. Recent evidence suggests that mfVEP can have clear role in monitoring and detecting progression of glaucoma based on good repeatability figures [19,159,170-172]. The important issue of early detection of glaucoma has not been settled and still needs further investigation. In some patients, the mfVEP will surpass the HFA in detecting early damage. In other patients, the reverse will be true. There are many examples where HFA and mfVEP are not consistent and do not agree in the

detection of visual field defects. Several authors [39,134,172] have shown that mfVEP detected damage missed by HFA. Further, abnormal mfVEPs have been reported in patients with normal HFAs. They attribute their findings to the subjective responses of the patient in SAP and the learning curve that can make small early defects missed by the patient. In contrast, Goldberg [134] reported that there is no learning curve for multifocal objective perimetry, make detection more reliable. He emphasized that in clinical practice many patients are unable to perform reliable subjective tests but can perform multifocal objective perimetry, and that many patients, particularly the elderly, have stated that they find this form of perimetry less stressful, because it does not involve decision making. On the other hand, visual field defects are well documented on the HFA but sometimes absent with the mfVEP. They attributed this to a possible effect of the stimulus location and its size. It is believed by the authors that the superior field will show smaller and irregular responses even in normal subjects. Similarly, subtle defects occurring in both eyes may be hard to detect. It is suggested by some authors [39,134,170,172] that mfVEP can be a suitable test for visual field assessment for those patients who are unable to perform HFA. Conversely, some patients can do better and perform more reliably in HFA and will have poor results in mfVEP. It seems at the end that the question is not which test is "better" in detecting glaucomatous visual field defects, but rather which test conditions will be more suitable for certain patients than others [39].

#### 1.3.2.2.8.2 Monitoring of glaucomatous visual field loss

As per the definition of glaucoma; it is characterized by the progressive loss of retinal ganglion cells producing very specific and characteristic optic nerve head damage and visual field defects. Since the publication of early studies within the area of visual field assessment, it was proven as stated earlier that the loss of ganglion cells takes place before it is manifested clinically or as a visual field defect. The role of standard visual field testing remained crucial in the detection and progression of glaucomatous field defects despite its variability and reliability issues. The shape and morphology of the optic disc is another variable factor that introduces additional difficulty to the initial assessment of glaucoma changes. For decades the full threshold HFA, and recently the SITA standard have been used to serve this purpose and provide clinicians with useful information that could be utilized in modifying management plans, and to monitor progression of the disease. With the rapid progression in mfVEP testing modification and adjustments of its protocols, high expectations were sought, especially as regards the early detection of glaucoma changes, where the role of standard visual field testing procedures is preceded by a significant level of ganglion cell damage. Due to its objectivity and reproducibility which has been shown in many studies [39,133,142,144,146,178] newer research work aimed at its role of early detection of glaucomatous visual

field defects. The mfVEP has shown significant inter-subject variability which arises from the differences in the visual cortex anatomical landmarks, such as the location of the calcarine cortex in relation to the scalp recording electrode montage. The major difference is in the pattern of local cortical folding within V1 [144,161,166]. Newer testing protocols and software reduced this variability from comparing the responses to similar corresponding points; either in the contralateral hemifield or the other eye [126,139,142,161]. One of the drawbacks of inter-ocular comparison (comparing responses in similar locations between eyes) is the possibility of bilateral glaucomatous damage. Thus the monocular mfVEP testing has arisen and developed in many studies [39,126,131,132,135,136,142,163-165] and more promising and less variable results were produced, especially the comparison with the use of SNR values. mfVEP has been used in clinical research on patients with various types of glaucoma to evaluate the glaucomatous functional loss objectively. Their results confirmed the ability and credibility of mfVEP test to detect glaucomatous visual field loss. Goldberg [134] used mfVEP test to detect glaucomatous visual field defects already documented in glaucoma patient. He found good correlation with the HFA field results in glaucoma patients, and reported that mfVEP can assess the visual field and identifies glaucomatous visual field defects, and that it may have the potential for identifying defects earlier than conventional perimetry. Graham et al [159] also reported similar results, and showed evidence that mfVEP is an effective method for detecting visual field loss in glaucoma. They suggested that the mfVEP test provides a valuable aid to the clinician in categorizing patients with unreliable, variable, unconfirmed, or excessive subjective field loss. However, the use of latency in mfVEP as a measurable parameter in the diagnosis and monitoring of glaucomatous visual field defects has been a source of controversy between researchers. While some studies, such as Parisi et al [70] reported almost 100% sensitivity and specificity of pattern-stimulus VEP latency in detecting glaucomatous visual field defects, other researchers [73,136] reported very small latency differences in fields with glaucomatous defects, indicating that latency is not a useful parameter in mfVEP assessment of glaucomatous visual field. When looking at many previous studies we can say that mfVEP signal amplitude has shown significant reduction or loss in glaucomatous visual fields [39,73,120,123,173-176]. But on the other hand, latency seems to be less sensitive, and this could be explained by the mechanism of cell death in glaucoma, where demyelination of optic nerve is not a feature or expected change in the course of glaucoma. This is in contrast to optic neuritis where marked latency delays are the main pathological change. Watanabe et al [173] also provided evidence that mfVEP can provide objective information about visual field in patients with hemianopsia. However, the authors found disagreement between the SAP and mfVEP tests in their subjects, but they considered the results of mfVEP were more reliable and useful. Based on their findings, they concluded in the case of occipital lesions mfVEP can be a useful tool in showing a

recovery of visual field defect even when SAP and mfVEP do not agree in results. Klistorner et al [125] reported similar results and showed evidence that mfVEP can be useful in detecting visual field defects due to central visual pathway lesions. In their study, they performed mfVEP tests for patients with various patterns of visual field loss due to central visual pathway lesions, including patients with known cortical lesions. They reported that the mfVEP can detect significant visual field losses arising from cortical lesions, but not with same accuracy in some cases of homonymous quadrantanopia, where the lesion may have been in the extra-striate cortex. They considered their findings as evidence supporting the concept that the mfVEP is generated in V1 striate cortex and that it may be able to distinguish striate from extra-striate lesions in patients with neurological deficits. Another study done by Greenstein et al [177] compared the results of standard HFA perimetry and mfVEP in detecting visual field defects in patients with strabismic amblyopia. They reported that both techniques were able to reveal deficits in visual function across the visual field in patients with strabismic amblyopia. In addition, mfVEP response latencies for amblyopic eyes were shorter than normal. They also found that mfVEP was able to detect field defects in the fellow eyes, which was not detected by HFA. It has been demonstrated that SAP is considered relatively insensitive to early glaucomatous changes, not just due to the early anatomical damage of ganglion cells, but mainly due to the poor level of reliability [179-181]. Recent studies suggested that glaucoma suspects, who are at risk for developing significant glaucomatous damage in the future, could benefit from mfVEP study, where the SAP results are not significantly showing any pathological changes. Thienprasiddhi et al [170] studied the ole of mfVEP responses, from eyes of patients with glaucoma suspect and those with ocular hypertension (OHT), in the evaluation of visual field defects when SAP results are normal. They performed the mfVEP test on three groups; normal controls, glaucoma suspects and ocular hypertensive patients. They found that mfVEP results were abnormal in 4% of the eyes from normal control group, whilst abnormal mfVEP were detected in almost 20% of the glaucoma suspect eyes, and 16% of OHT eyes. They concluded that the new mfVEP technique using monocular strategy with SNR comparison can detect visual field defects in a minority of eyes with glaucomatous optic discs and normal SAP results. Graham et al [159] also evaluated the role of mfVEP in glaucoma practice. They tested 436 patients who were referred for glaucoma assessment, where they all had mfVEP test done. They found that the results of the mfVEP correlated well with the stage of the glaucoma and the Humphrey mean deviation, with an overall sensitivity for detecting glaucoma of 97.5% for established defects, and 95% for early defects. They reported that 92.2% of low risk suspects had normal mfVEP. They concluded that mfVEP is an effective method for detecting glaucomatous visual field defects, and that it provides the clinician with useful information about the visual field when there is also an unreliable SAP test. Goldberg et al [134] also found similar results when he compared the results of

HFA and mfVEP done for glaucoma patients. They reported that mfVEP was able to detect abnormal visual field defects in 50% of patients were reported as normal using the SAP test. Other studies [19,160,171,172] have shown similar results and provided evidence that mfVEP can detect early glaucomatous visual field defects in glaucoma and glaucoma suspect patients. These results were not opposed by recent studies. The majority of studies identifying the role of mfVEP in detection of glaucomatous visual field defects confirmed its ability to detect already existed damages with high sensitivities ranging between (86%-97.5%) and for the defects that not detected by SAP with lower sensitivities (76%-92%), which is a very good detection rate compared to the SAP results and its limitations in the early stages of glaucoma [145,159,161,171,179-181]. Boonchai et al [182] proposed a statistical method for monitoring progression of glaucoma using the mfVEP. They tested two groups of glaucoma patients; the first were retested after 50 days to check repeatability, and the second were retested after one year to check for progression. The data from the two tests of the two groups were compared. They reported a good level of repeatability of the first group with no significant changes, a significant level of progression in the second group compared to the standard SAP test. They concluded that the cluster analysis protocol they created could be used as a method for assessing progression of glaucoma using the mfVEP technique. As a theoretical framework for judging whether SAP or mfVEP can be more beneficial, Hood et al [39] provided a better understanding of the two tests based on comparison of matched probability plots between the two tests. They concluded that in the majority of the time the mfVEP can detect glaucomatous visual field defects earlier than SAP. However the reverse can occur as well, assuming that SAP results are reliable. Their analysis suggested that the two tests will often agree. But regardless of the accuracy of mfVEP results and its ability to detect visual field defects earlier, they predicted that it will never replace SAP tests in the near future. There are significant reasons made the use of mfVEP as a primary tool for objective visual field testing limited. The test is lengthy specially in the two runs mode, which is the one used for diagnosis and monitoring, the equipment is expensive compared to conventional HFA and other machines, performing the test needs qualified and well trained technical staff who can connect the electrodes accurately and monitor for any intra-test errors. Despite this, many subjects prefer the mfVEP test over the standard HFA because it is less dependent on patients' responses. For clinicians, it cannot be performed for all glaucoma patients on a day-to-day practice because of its lengthy testing duration. The interpretation of mfVEP test results is another limiting factor, as it requires the clinician to possess a good knowledge of VEP testing and potential sources of testing error. All these factors have put the mfVEP test behind where it should be as a highly sensitive and repeatable objective perimetry testing tool.

#### 1.3.2.2.8.3 Ruling out non-organic visual loss

Like conventional VEP, patients with non-organic visual loss will typically have normal mfVEP responses. This has been shown in one study [183] where they found that mfVEP can detect a non-organic overlay of visual defect that can be missed in a routine eye examination or overlooked during the analysis of an unexplained visual loss. It can also draw a picture of how the patient's visual field looks like when a non-organic cause is suspected. In those patients the mfVEP can confirm the extent of any visual field defect was previously exaggerated in its extent or depth when tested by HFA [71].

#### 1.3.2.2.8.4 Diagnosing and monitoring of optic neuritis

The role of mfVEP in the monitoring of optic neuritis can sometimes be more advantageous than conventional VEP, especially in the recovery phase, when the patient regains central vision but still have qualitative visual complains sometimes described as haziness [160,184]. The authors explained their findings as mfVEP could have the ability to detect local demyelination or focal delayed healing in the optic nerve. The superiority of mfVEP over VEP arises from the limited function of the conventional VEP in detection of visual field defects, as the conventional pattern VEP is an averaged waveform summing the responses from the entire tested visual field unequally. Thus the final VEP output will even out all response variability from different field locations, and is influenced by the degree to which normality outweighs or masks the abnormal focal locations. For these reasons, the authors suggested that the use of mfVEP might have the advantage over both conventional VEP and standard HFA in following patients with optic neuritis [183,184]. Moreover, mfVEP testing also showed good promising results in the assessment of neurological diseases complicated by mental disorders. In one study [185], the authors used the mfVEP in the assessment of neurological diseases and they concluded that the mfVEP objective assessment of the visual field is a useful tool in patients with intracranial diseases complicated by mental disorders, where the mental disability renders them unable to perform subjective SAP. Frazer et al [186] showed evidence that there is a role of mfVEP test in the assessment of demyelinating optic neuritis, such as multiple sclerosis (MS). They concluded that the mfVEP test is a sensitive and specific tool for detecting optic neuritis. They found a significant difference in latency analysis findings between MS patient groups. The Latency results suggested in their analysis a role in identifying a patient's risk for future MS. In a later analysis for the same authors [186] they analysed the latency and implicit time of mfVEP waveforms in patients with MS and found statistical difference in latency over one year period. They considered that this evidence may indicate that mfVEP latency delay can assist in predicting progression to future MS.

#### 1.3.2.2.8.5 Confirming unreliable visual field tests

Some patients will have unreliable visual field tests due to variable reasons; it could be related to the reliability indices or the test variability itself. In these situations mfVEP can provide objective visual field assessment at a good level of sensitivity and specificity. It has been well known that taking a visual field test on the standard HFA system has been very difficult for children, who show an almost constant high level of unreliability. mfVEP could be a very good tool to assess the visual field in children where no responsive actions or inputs are required from the child to get good and reliable results [187].

### 1.3.2.2.9 Repeatability of mfVEP compared to HFA

The ability of the test to provide credible information about the visual field is essentially controlled by its ability to show good repeatability scores. Decisions related to management are mainly depending on the good sensitivity and specificity figure of the test, and its ability to show consistent results over time. One reported problem that affects the ability of SAP tests to determine progression of glaucomatous visual field defects is that the measurements become less repeatable as the disease advances and defects increase in depth or size [10,17,189,190]. In response to this shortcoming in progression monitoring major improvements in the analysis software led to more accurate threshold detection and the introduction of new methods of perimetry algorithms that improved the progression analysis of SAP tests [191-195,197]. However, despite the improvements over the past two decades, the debate still presents about the reliability of SAP in general, and with the introduction of mfVEP, the question extended to include whether mfVEP as an objective test could offer more reliability figures, and thus improve the repeatability of visual field assessment. mfVEP has shown in many studies a good level of repeatability among normal subjects, and glaucoma patients, in short and long reassessment intervals. For example, Goldberg et al [134] evaluated the mfVEP responses in controls and glaucoma patients on two separate occasions and they found no significant differences in the responses across the field. Klistorner et al [146] reported the same results after comparing the two separate tests done in few days interval. Later, Klistorner et al [196] measured the coefficient of variation between two successive mfVEP tests in two groups, and reported a low value (16%) confirming a good level of repeatability of the mfVEP test. Other studies compared the repeatability of the mfVEP tests compared to the standard HFA results, such as Chen et al [145] who compared the repeat reliability of both mfVEP and HFA tests in a retrospective study and reported a better repeatability of the mfVEP test, where the signal amplitudes were more reliable than the SAP thresholds. Meanwhile, Bjerre et al [198] reported a similar degree of repeatability of SAP and mfVEP in his work measuring the test-retest reliability. They found that both tests showed a large degree of test-retest variability in the

number of defective test locations. They recommended modifications of the processing time of mfVEP signal to remove the relationship between the number of defective locations and signal amplitude. They also reported that the majority of patients preferred mfVEP to conventional perimetry although mfVEP takes longer to perform. More recently, Fortune et al [119] evaluated the repeatability of the mfVEP test and compared it with the SAP repeatability parameters in a group of normal subjects in two separate occasions one year apart. They found that the repeatability of mfVEP was slightly better than SAP visual field results after one year of retest. Based on their findings they suggested the use of mfVEP in early progression stages of glaucoma as it would be much easier to detect glaucomatous visual field defects. They also reported some limitation in the peripheral locations of mfVEP test, where the low dynamic range of SNR calculations made the detection of progression in these locations more difficult and unreliable. From the previous results it is evident that mfVEP repeatability is at least equal to the standard SAP results, if not better in some research work, which makes the test beneficial at the early stage of glaucoma diagnosis and as a follow up tool to monitor progression.

### 1.3.2.2.10 Comparison of mfVEP and HFA topographies

Both mfVEP and SAP strategies test the same visual field using different techniques and probability maps. These techniques and more importantly the probability maps, do not match in their spatial resolution or in the interpretation of their meanings. The tested visual field is sampled in a very different way by the two techniques. Within the central 2.61 degrees (5.21 degrees diameter) there are 12 mfVEP responses covering this location, while only one HFA test point is covering this area in the standard 24-2 protocol. Conversely, in the outer testing rings, three or four HFA test points fall within each of the individual segments. The conclusion is that for two differently scaled tests spatially it is difficult to be sure which points can be corresponding to its similar region in the other test. There is clearly a large range of amplitudes for the normal controls [39,76,77,133,145,159,199,200]. This normal range includes very low amplitude responses in some locations, but larger amplitudes in other locations, which do not reflect the same retinal sensitivity distribution seen in SAP results [77,200]. As a result of this mismatch, it is sometimes difficult to distinguish what is normal from abnormality using a single test, especially clearer in the case of mfVEP. The idea of comparing the results of the two tests is sound and valid, and definitely will add new information about the assessment of the visual field, assuming that the two tests are compared accurately, considering the important differences between them in spatial resolution. When comparing the probability plots, the two tests will be scaled according to different systems covering the same range of visual field in almost the same degrees. However the end results of the two scales can be compared directly, which is the method used to compare the tests in many

studies mentioned earlier in this review. But, there are some qualitative differences related to the locations of the different stimuli. For example, in mfVEP defects are difficult to detect in the superior field, as the responses from normal controls are so small. On the other hand, a defect in the inferior field appears in the mfVEP plot, but not in the HFA 24-2, which indicates that detecting normal low amplitude (small responses) shows more variability between the two tests [39].

### 1.3.2.2.11 Limitations of mfVEP

There are some limitations that make the mfVEP test either unreliable or unobtainable. These limitations could be related to technical (operational) or test-taking errors. The technical part include limitations that can also be found in standard visual field testing; such as the eyelid occluding part of the field, correction of refractive errors can sometimes give false results [126,142], clarity of the media is important [39,161], and the excessive eye movements. Eye movements especially could be a very important confounding factor as it is in the visual field test. The eccentric fixation can give falsely higher level of response for far peripheral points. Conversely, the unsteady fixation can give diminished responses in the centre of the field as reported in some studies [71,132,201]. Other limitations are related to the nature of the test and how it is testing the field responses. In the periphery the spatial resolution is very poor, thus the responses are almost always less reliable with much higher level of noise [39,160]. The sectors at the periphery are in some locations over $7^{o}$ in width, leaving large spaces in the peripheral field as "untested" or at least without a recordable response. In this case it is difficult to establish a diagnosis as a confirmed visual field defect considering only one response from such a large area, two or three adjacent points with individual responses would have been more confirmatory as proved before in many studies [71,126,134,161,202]. Detection of the blind spot is also difficult in most of the mfVEP recordings because the spatial resolution of the test can easily overlook reasonable large, focal defects if they are restricted to the rings beyond the central $15^{o}$ which is the case in the blind spot as a physiological focal field defect [39,71,160]. Poor perimetry due to patient unreliability or level of cooperation is not an uncommon problem in mfVEP testing. The test itself seems very lengthy and inconvenient to some test-takers, even if they are healthy normal subjects. Patient inattentiveness is another problem that can infect the VEP waveforms with alpha waves and sleep spindles [160-162]. In addition it has been shown that poor visual field test subjects are good mfVEP test subjects [71], meanwhile there are some good visual field test subjects who are unable to perform well in a mfVEP test and their results either poor or significantly variable. Some of these limitations can be handled easily by good instruction to the patient, monitoring of excessive eye movements through small camera, dividing examination time into small cycles with reasonable rest in between, and good equipment calibration. Other types of errors and limitations are not correctable, especially

those related to the spatial resolution of the test. However, current and future research work should offer new ideas about how to represent the visual field in mfVEP testing in much smaller point spacing. The mfVEP test duration can be considerably longer than the standard HFA method, especially if the SITA standard test is used, which is significantly shorter in time than the full threshold strategy. Fortune et al [133] evaluated the potential effect of reducing the recording duration, from 16 minutes (2-run test) to 8 minutes (1-run test) per eye, on the diagnostic performance of the mfVEP in eyes with high risk ocular hypertension and early glaucoma. They found that there was good agreement between the two versions of the test, with good diagnostic performance of the two tests. However, sensitivity of the 2-run test was higher. They concluded that if high sensitivity is required in a group of high risk patients then the best approach is the 2-run lengthy mfVEP test, as this test showed better discrimination between eyes. But if high specificity is needed then the 1-run (shorter) version could be a good and adequate option to facilitate the test at an accepted level of accuracy. The long duration test still, whether it is 8 or 16 minutes per eye, a lengthy testing procedure for most of the patients.

#### 1.3.2.2.12 The need for combined structural and functional tests

The use of functional visual field assessment has been the gold standard method for detection of field scotomas and defects for decades. Despite its shortcomings and inconvenience to many patients, it stayed on top of routine tests for glaucoma assessment till today. The introduction of accurate anatomical assessment of glaucoma changes, specially the thickness of the retinal nerve fibre layer, was a breakthrough in the early detection of nerve damage. Clinically, it would be extremely helpful to have an agreement between a functional assessment of the visual field, such as HFA or mfVEP, and structural assessment of optic nerve head and retinal nerve fibre layer thickness. The combination between these tests can overcome the shortcoming of each individual test such as poor reliability or the SAP learning curve. Many studies have shown a significant correlation between the visual thresholds recorded by SAP tests and the retinal ganglion cell density that confirmed by histological cell analysis [203-205]. In the early stages of glaucoma, where selective types of ganglion cells are more affected and first to be lost, SAP assessment is insensitive to this selective loss since the response of other types of ganglion cells mask a defect [203,204]. The need to combine two tests; functional visual field assessment and anatomical changes in the optic nerve thickness, has become a source of debate until today. Numerous studies [206-211] have shown a correlation between significant optic nerve morphology changes, nerve fibre layer thickness loss and the standard visual field parameters in documented glaucomatous visual field defects. In these studies, they recommended using a combined strategy of structural and functional tests in the diagnosis and monitoring of glaucoma based on the correlation between the

two. Recently, Bouzkurt et al [212] evaluated the relationship between global indices of Humphrey SAP central 30-2 threshold test using SITA standard strategy, Humphrey Matrix 30-2 frequency doubling technology (FDT) threshold test, and HRT II parameters in ocular hypertensive (OHT) and glaucoma patients. The study group also tested other parameters and factors, like the level of agreement among the 3 groups specially in classifying study groups into normal and abnormal. They also evaluated the correlation between specific sector rim loss and its detection by other functional visual field assessment tests. They found that visual field global indices showed statistically significant correlations with most of the rim changes detected by HRT parameters. They suggested based on their findings that the use of either HRT or standard visual field assessment tests may show the first evidence of glaucomatous damage. They recommended the combined use of both tests to assess the optic nerve head analysis and visual field results in order to obtain better detection of early glaucomatous visual field defects. Similar study compared the sensitivity and specificity of the HRT with mfVEP as two objective visual field tests, done by Balachandran et al [213], they compared the data of functional and structural assessment where they found that both of them can detect existing glaucomatous visual field defects with limited correlation, ranged between 50%-77% depending upon the type of parameter used for comparison. Sensitivity and specificity of mfVEP were higher compared to HRT (93%, 96% - 79%, 92% respectively). They concluded that the mfVEP correlated better with the HFA than with HRT. However it remained important in their recommendations to use both functional and structural assessment for best monitoring of glaucomatous visual field defects. In a very recent study, Moschos et al [214] also evaluated the anatomical and functional changes of optic disc secondary to glaucoma by using a combination of mfVEP and optical coherence tomography (OCT). They tested two groups; normal controls and glaucoma patients who underwent the two tests. They found that the combined use of these two tools enhanced the ability to detect the glaucomatous visual field defects, and they suggested that it is useful to diagnose and monitor glaucoma using this combination.

# 2.0 METHODS

## 2.1 STUDY SITE AND SETUP

This study was conducted at the ophthalmology outpatient clinic, Hamad Medical Corporation, Doha, Qatar. Study subjects were recruited and selected from patients attending the ophthalmology outpatient clinics during the recruitment period, which was between March and June 2012

## 2.2 OBJECTIVES

The purpose of this study is to examine the effectiveness of a new analysis method in the mfVEP test when it is used for objective assessment of visual field in glaucoma. The hemifield sector/hemi-ring analysis (HSA) compares the responses of each sector and hemi-ring in one hemifield of visual field to its fellow corresponding sector in the opposite hemifield. In addition, each sector and hemi-ring in any hemifield is compared to its similar ones in the same hemifield between the 3 study groups. The results of this analysis protocol were also compared to the SAP technique. It is assumed that the protocol provides good sensitivity and specificity levels in the detection of early visual field losses and the detection of existing significant glaucomatous visual field. Specific study aims are listed:

1- To measure the retinal responses in central 48-56 degrees of the visual field in all study groups carried out by:
   a- Standard SAP visual field testing for central 24-2 using the SITA standard strategy.
   b- mfVEP objective perimetry technique using the HSA protocol.
2- To compare the results of the two tests in all study groups, to determine whether new analysis protocol can detect early changes in visual field that was not detected by conventional automated perimetry methods

## 2.3 SUBJECTS

Three study groups of participants were recruited; two study groups and one normal control. Group 1: Patients with known diagnosis of glaucoma who may or may not be receiving treatment. Group 2: Glaucoma suspect patients who have one or more risk factors for glaucoma but do not have established clinical damage to the optic disc or visual field losses. Group 3: control group of normal subjects for comparing their results to the two study groups. Glaucoma patients were enrolled to evaluate the ability of the HSA analysis protocol to detect an already documented visual field defect and to what degree. Glaucoma-suspect patients usually have one or more risk factors of glaucoma but without apparent optic disc damage or true and reproducible visual field defect detected by SAP test, but they are considered more liable than others to develop glaucoma

in the future. Glaucoma suspects were included into this study in order to detect any differences between the SAP testing and mfVEP perimetry results in early detection of functional visual field loss secondary to glaucoma changes. Benefits of this study lie in the ability to utilize this analysis protocol in a larger scale and to extend this to other patients to help detect glaucoma damage at an early stage.

## 2.4 RECRUITMENT

All study participants were recruited and selected from regular patients, patients' relatives attending the ophthalmology outpatient clinic for scheduled eye care services, and normal volunteers. Participants were allocated to each study group according to their criteria after approval. The study recruitment procedure involved direct contact with potential participants in the waiting area, in the examination rooms at various ophthalmic clinics, and at the ophthalmology investigative unit. A group of well-trained research assistants and certified ophthalmic technicians were responsible for direct communication with potential participants, offering to join the study. The selection of study participants was random according to the selection criteria. All study subjects gave written informed consent before enrolment in the study. All testing procedures, risks and expected side effects were discussed and explained to study subjects at the time of giving consent. The protocol for this study was approved by the Aston Audiology/ Optometry Research Ethics Committee (AOREC), Aston University, and the research ethics committee of Hamad Medical Corporation (HMC) Doha, Qatar. All consenting procedures followed the tenets of Declaration of Helsinki.

## 2.5 PRETEST ASSESSMENT

All study subjects had a full eye examination completed prior to enrolment by a glaucoma specialist; including intraocular pressure, Gonioscopy, visual acuity using Snellen chart, slit lamp assessment of the anterior segment, and retinal examination including the optic nerve head (optic disc). All candidates were found to be medically fit, were not taking any medication known to influence the visual field or results of mfVEP, and matching our inclusion criteria were offered the chance to participate and take the study tests.

## 2.6 SELECTION CRITERIA

### 2.6.1 Inclusion criteria

Selection criteria for glaucoma and glaucoma suspect patients followed the guidelines of the American Academy of Ophthalmology Preferred Practice Pattern (AAO-PPP) in the precise

definition of glaucoma and glaucoma-suspect patients [7]. Glaucoma patients group included any patient with already diagnosed and well documented features of any type of glaucoma as defined by the AAO-PPP guidelines, including:

1- Patients with evidence of optic nerve damage showed by neuroretinal thinning associated with cupping or other signs of glaucomatous optic disc changes.
2- Reliable and reproducible glaucomatous visual field abnormality consistent with ganglion cell damage, showing significant difference between the two hemispheres, in the absence of any other causes or explanations.

A glaucoma-suspect patient is defined as an individual with clinical findings and/or constellation of risk factors that indicate an increased likelihood of developing glaucoma, including any patient with a well-documented risk factor of glaucoma without any anatomical or functional damage to the optic nerve as defined by the AAO-PPP guidelines [7]. The glaucoma risk factors considered include:

1- Suspicious appearance of the optic disc such as enlarged cup–disc ratio, asymmetric cup-disc ratio, notching or narrowing of the neuroretinal rim, disc haemorrhages, nerve fibre layer defect.
2- Suspicious visual field for glaucomatous damage in the absence of clinical signs of other optic neuropathy, such as arcuate bundle defect, nasal step, paracentral scotoma, altitudinal defect, larger mean pattern standard deviation.
3- Consistently elevated intraocular pressure associated with normal appearance of the optic disc and retinal nerve fibre layer and with normal visual field test results.

Normal subjects were recruited after excluding any existing ocular pathology or other factors that can affect the visual field, to set a cut-off level for normal values for each test.

## 2.6.2 Exclusion criteria

The recruitment for this study was restricted to general eligibility criteria that were applied to all study groups. Subjects suffering from visual field losses due to any pathology other than glaucoma, significant retinal disease with or without macular involvement, established neurological deficits that affect visual cortex or visual pathways, subjects receiving medications with known effects on the visual field, or subjects with amblyopia were excluded from joining this study. Subjects under the age of 16 were not recruited for this study; due to the high level of variability and poor reliability exist in SAP test for patients under age of 16 [5,48,58]. Vulnerable subjects suffering from mental or physical disability, and/or severely debilitated patients who need continuous and special care were excluded from this study, as high level of cooperation and responsiveness are both needed to perform study tests. Both genders were equally invited to participate in this study.

There was no language restrictions applied when subjects were recruited. Arabic as a main language, English as an international language and other commonly presented languages in the clinics were handled efficiently by multilingual technical staff.

## 2.7 SAMPLE SIZE

We used the sample size estimation for bivariate correlation coefficient tables to calculate the appropriate number of subjects in each group to acquire a significant level of correlation between the two tests; the standard SAP and mfVEP. Many previous studies [39,133,142,144,146,178] have shown a range of significant correlation coefficient (r) ranged between 0.69-0.86. The sample size was estimated according to a preset power of 0.9, Beta level of 0.1, Alpha level of 0.05 and at a desired correlation coefficient (r) level of 0.72, which is the median (r) value of previous similar studies. Sample size for each group is 16 subjects. All calculation were implemented using MedCalc© software (Version 11.5.1 bvba, Broekstraat 52, 9030 Mariakerke, Belgium).

## 2.8 THE MFVEP TEST

### 2.8.1 Stimulus

According to the guidelines of the International Society of Clinical Electrophysiology of Vision (ISCEV) [215] there is a standard requirement for the multifocal stimulus. These standards were fulfilled using a CRT frame frequency of 75 Hz, which has been used widely in other studies [19,39,71,132,134,136,160,161,185]. For CRT displays, the stimulus luminance elements were in the light state 100 cd/$m^2$, and the dark state was a Michelson contrast of ≥ 90%. Standard m-sequence was used to control the temporal sequence of change between the light and dark stages of each stimulus hexagon. This m-sequence, in which each hexagonal element can change with every frame in a pre-set and calculated (pseudorandom) order, is the standard for routine testing. Central fixation was achieved by central patterns or dots of variable sizes, which is commercially available in the Roland Consult system used in this study. The pre-processing filtering is accomplished by 6 channels amplifier and by the system filtering processing. For a basic mfVEP, the band pass of the filters should be approximately 5-200 Hz. The acceptable range for the high pass cut-off is 3-10 Hz and for the low pass cut-off is 100-300 Hz. Most of the mfVEP recordings to date have been obtained using a dartboard pattern like the one shown in (figure 10-A&B). This pattern is a standard option (Dart Board 58 with Pattern segments) of the Roland Consult GmbH software, RETIscan (Brandenburg, Germany). A modified version of the analysis software has recently been introduced as part of the hemifield sector analysis protocol software Roland perimeter version 6.1.4.11. The modification was based upon the new idea of this research work to record the SNR

values of each segment (figure 11-A), then calculating the average of individual sectors that contains a fixed number of segments as shown in (figure 11-B). Each sector SNR value in one hemisphere is compared to its corresponding sector in the other hemisphere to calculate the difference between the two fellow sectors. Similar averaging and calculations were carried out in a hemi-ring group of segments arranged circumferentially around the central part as shown in (figure 11-C&D). SNR values from each two fellow hemi-rings are compared to see if there is any statistical difference between each couple of sectors / hemi-rings in the 3 subject groups. The dartboard pattern was presented on a monitor viewed at a distance of 30 cm; the diameter of the display subtended 44.51 cm. There are 58 segments in this display and each segment contains 16 checks, 8 black and 8 white. The dartboard pattern covers a central $25^{O}$ on each side of fixation point with nasal extension wing up to $42^{O}$. The segments, and the checks, are scaled to be of approximately equal effectiveness, based upon cortical magnification factors presented by Baseler et al [(162)]. For example, the innermost sectors are about 3 cm (1.21 inches) width, while the outermost sectors exceed 18 cm (7.1 inches) width. The 58 mfVEP segment responses can be clustered and arranged in groups for display purposes and to easily calculate the SNR values assuming that there is no much loss in spatial resolution.

## 2.8.2 Technical specifications of the test procedure

Each participant underwent two HFA 24-2 SITA standard tests with one hour apart during their enrolment period. Patients were instructed in a clear and informative language on how to take the HFA tests effectively without errors. One or both tests could have been repeated on individual basis if the results were not accurate or if it contained high level of errors. On completion of perimetry, both pupils were dilated using a topical mydriatic (Tropicamide 0.5%). When both eyes were fully dilated the patient was prepared and connected to take the second test, which is the multifocal visual evoked potential objective perimetry test (mfVEP). During the mfVEP test, the subject was asked to focus on a screen that projects flickering hexagon patterns on the patient's eye. Total 2-run test time is about 16 minutes per eye, during which; the examiner was monitoring the patient's eye to observe any unwanted eye movements, which could affect the results. However, those subjects who failed to take any of the tests effectively had to retake it at a later time. Test was counted as "not valid" if it contained too much noise or errors. All data obtained from the 3 study groups was analysed using the new Hemifield Sector Analysis protocol (HSA). mfVEP data from the 3 study groups were compared to the SAP test results to check for significant difference or agreement. The final outcome of comparison and evaluation of the data confirmed or rejected the validity of the new analysis protocol in detecting visual field defects. Total duration of attendance for each subject ranged between 90-120 minutes, depended on the individual

variations among subjects in test taking. No follow up visits were required; all recordings were taken in a single session. Data of the two tests were compared in all study groups to identify whether the new hemifield sector analysis protocol has the ability to detect documented visual field defects, the ability to detect early visual field losses when it is not detected by standard SAP testing.

### 2.8.3 Electrode montage

The electrode montage used in this study was a bipolar saddle-shaped connecting 4 electrodes to the scalp at the occipital cortex. The 4 electrodes were touching 4 points around the inion as shown in (figure 12). These 4 electrodes, horizontal and vertical orientations, give rise to 4 different channel recordings around the Inion to maximize signal detection. The recordings from the 4 channels were compared through the software and the best signal (best SNR value) was chosen to represent the response from each segment.

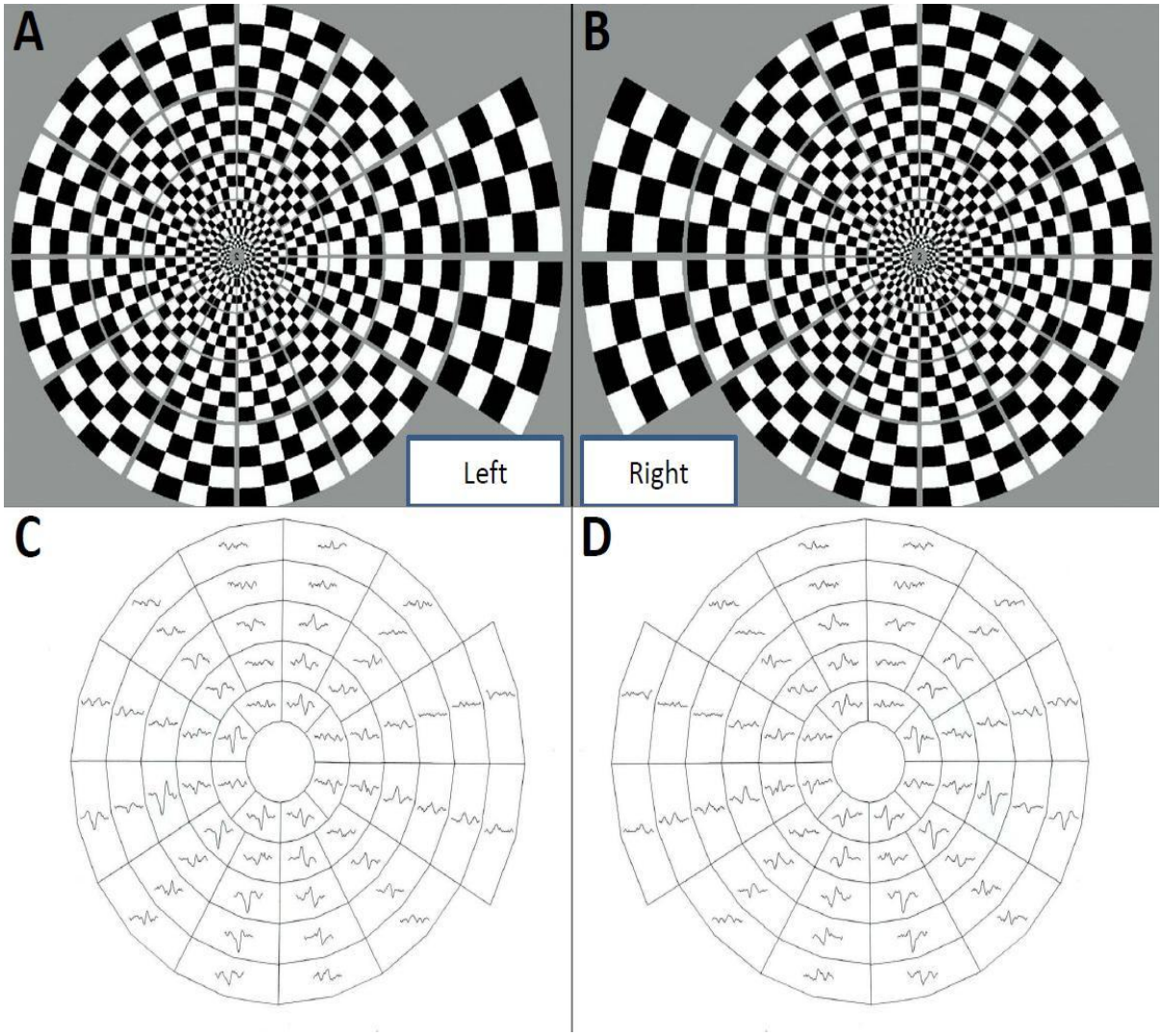
### 2.8.4 Recording system

The RETIscan system manufactured by Roland Consult, Germany was the system used for performing all mfVEP tests for this study. (Figure 13) shows the standard connections and amplifier used in the system in addition to the specifications of the testing procedure. All participants were optimally refracted for near vision. An appropriate trial lens was placed in front of the patient's eye during the test for optimal visual acuity in the near distance. Patients were seated 30 cm from the screen, and instructed to stay focused on the central target and not to move their eyes during the test.

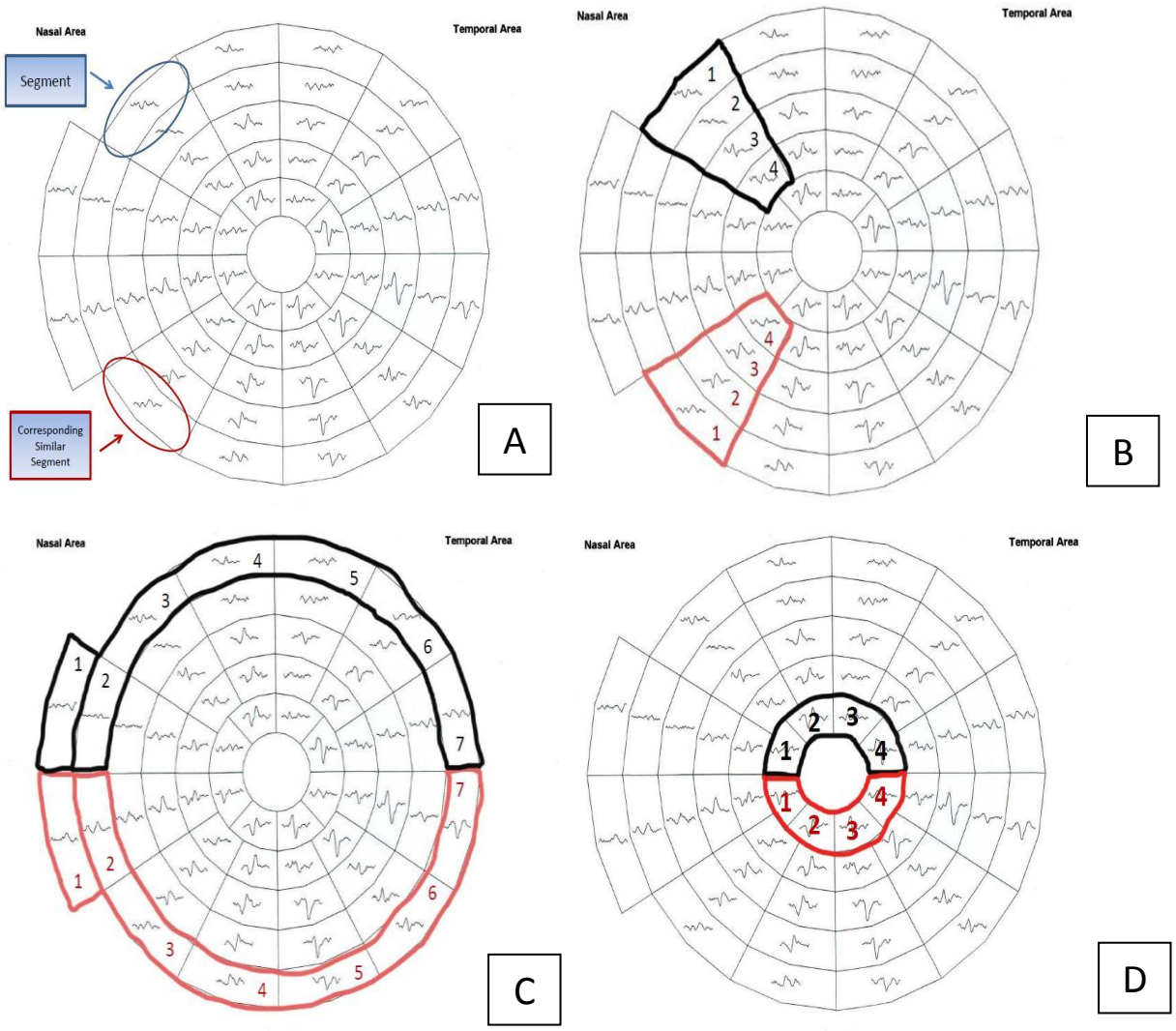
### 2.8.5 mfVEP analysis protocols

Many mfVEP analysis protocols were tested and have shown good correspondence with clinical data and standard SAP results in glaucoma patients. However, objective early detection of mild changes in the patient's visual field, while these defects are not large enough to be observed by the patient or clinically, still controversial. It has been well established that conventional visual field testing using SAP protocols almost always influenced by intra and inter-test variability related to the testing strategy itself, or the physiological fluctuation in retinal sensitivity in glaucomatous visual field defects [3-5,11]. mfVEP testing also shows different sources of variability, which could be attributed to the test setup, machine calibration, connectivity, electrode and stimulus montage, and individual anatomical variation of convoluted cortex in relation to external scalp recording electrodes [39, 134,166]. Moreover, It was reported [1,2,166,167,203,204] that at least 25-50% of the ganglion

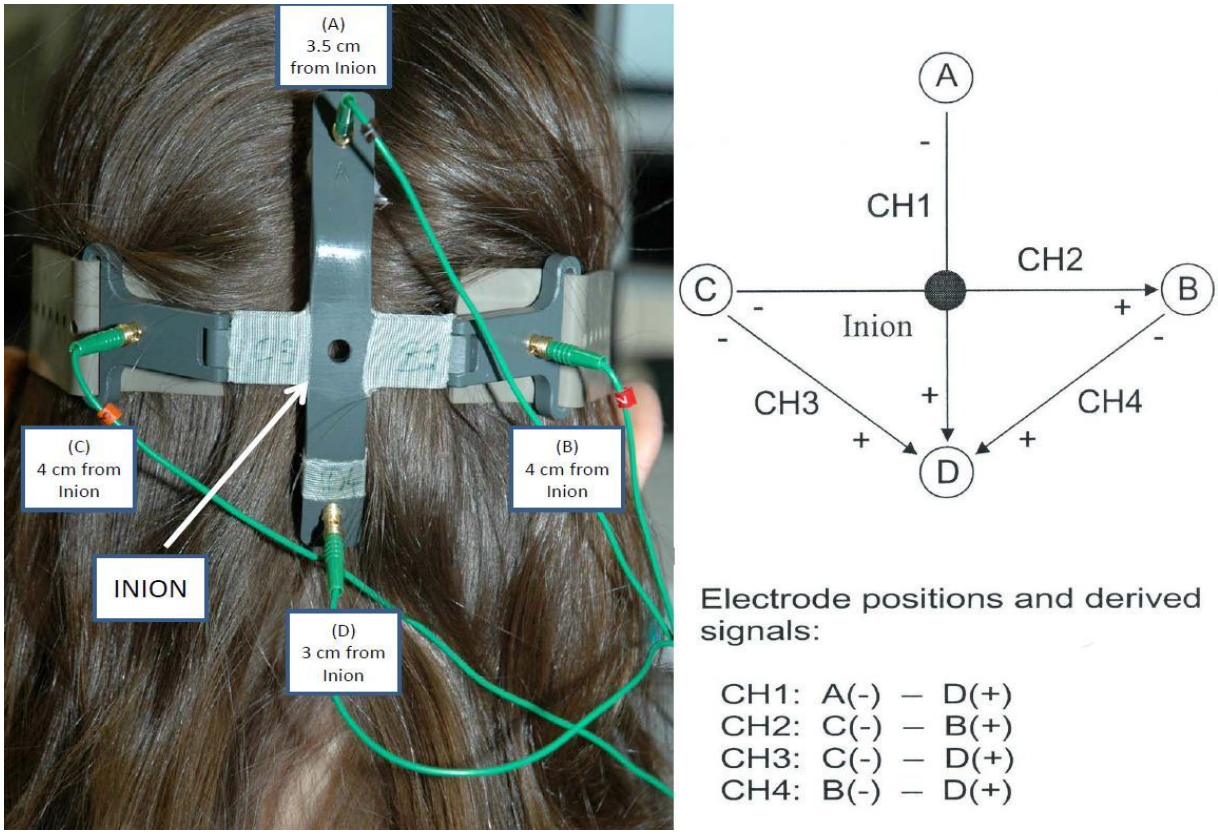
cells must be lost prior to the development of a field abnormality detectable with modern automated visual field testing. The Hemifield Sector Analysis (HSA) protocol used in this research is novel, simple and following well-known anatomical and physiological facts. These are: 1- The visual field is identically divided along the horizontal meridian into superior and inferior hemispheres, following the distribution of Nerve Fibre Layer (NFL) in the innermost part of the retina. 2- According to the pathophysiology of glaucoma; damage usually starts in any sector at one hemisphere before the other corresponding one within the same eye. Thus, and according to these facts, functional analysis of early changes in one sector could be theoretically detected when compared to its corresponding sector in the fellow hemisphere, which is the same concept currently employed in the Glaucoma Hemifield Test (GHT) analysis in SAP testing. This research postulates that the HSA protocol can have good level of sensitivity and specificity compared to standard SAP, in the detection of glaucomatous visual field defects, and can provide better detection ability to small defects. This approach has not been tested or evaluated before. A similar approach that has been previously investigated is the Inter-Eye Asymmetry Analysis [196], where the authors compared each mfVEP field segment in one eye to its corresponding segment in the neighbouring eye. They were able to extract a Response Asymmetry Coefficient (RAC) marker used to detect field defects in glaucoma. Other studies compared individual segment responses to a pre-set range of normal values extracted from previous studies. Some analysis protocols compared mfVEP data to SAP results and concluded that objective perimetry results are comparable to those of SAP. This study used each hemisphere as a reference for its identical corresponding one within the same eye, in order to maximize the validity of comparison. Currently, the gold standard method used for detection and monitoring of visual field defects in glaucoma is the SAP testing protocols. Objective perimetry is a new concept that has been heavily studied since its introduction in 1992 [123]. The testing protocol itself has gone through series of modifications trying to acquire the most accurate recordings with fewer errors and more reproducible data [39,161,144,146,196]. The most recently applied and widely accepted testing protocol is the one used in this study; which is based on a cortically scaled stimulus, multifocal, pseudorandomly alternated dartboard in the shape of small hexagon pattern stimuli testing the central 25 degrees on each side of fixation, with a nasal wing extension up to 42 degrees [144,146,196]. Most of the suggested analysis protocols used either “Ring Analysis”, which is the average of individual amplitudes in all tested segments located within each circumferential ring across the tested visual field. Another accepted and established analysis protocol is the “Quadrant Analysis”, which is the average of signal amplitudes in all tested segments located within one quadrant of the tested visual field. The patient’s own responses were used as a standard reference for comparison, which is expected to be a more reliable approach.




(Figure 10) Standard mfVEP dartboard pattern-reversal stimulus covering central 25° with asymmetric nasal wing extending up to 42° (A,B). Standard mfVEP results printout showing traces plotted in each segment in both eyes (C,D). *Author's copyright.*




(Figure 11) Shows the 58 segments of the right visual field. The field is divided into two identical hemifields across horizontal meridian; each segment has a similar correspondent in the opposite hemifield (A) SNR value is calculated in each segment. The average SNR of wedge sectors (B) and semi-circular sector; peripheral and central (C,D) are calculated to compare its values to the fellow corresponding sector on the opposite hemifield. *Author's copyright* .



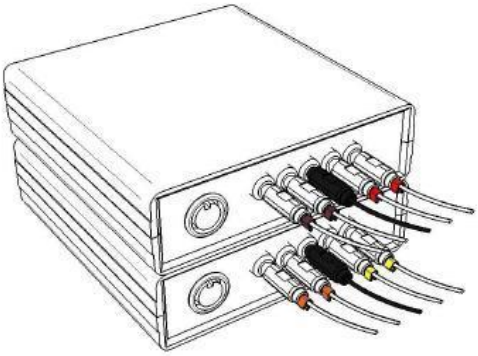

(Figure 12) The occipital straddle with vertical and horizontal channels mounted on the scalp with the Inion as the main reference point for all connections made by Roland Consult – Germany (Left). Bipolar Occipital Straddle (both vertical and horizontal electrodes) record the best signal in 4 directions; two oblique, one horizontal and one vertical, reflecting cortical activity in 4 locations around the Inion. The introduction of this straddle improved the signal especially in the horizontal meridian, it reduced variability and recordings showed remarkable similarities in the waveform between the two eyes in normal subjects, which helped in standardizing this electrode montage to obtain optimum reproducibility. *Adapted from the Roland Consult manual.*

**Amplifier**

Please connect the VEP cable to the corresponding channels. All amplifier cables are marked with the channel number.

| Color | Channel |
|---|---|
| Brown / 1 | 1 |
| Red / 2 | 2 |
| Orange / 3 | 3 |
| Yellow / 4 | 4 |

The two remaining black cables have to be connected to the amplifier's ground connectors.

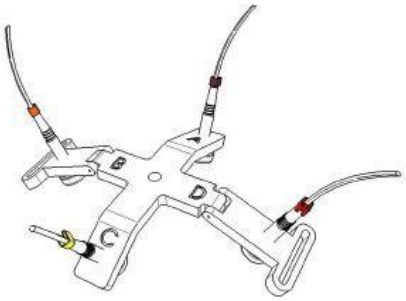


**VEP-Cross**

The green electrode cables for the VEP-Cross are marked with same colors. Please connect it as it is shown in the following table:

| Color | Position |
|---|---|
| Brown / 1 | A |
| Red / 2 | D |
| Orange / 3 | B |
| Yellow / 4 | C |

The hole in the middle of the cross has to be placed above the inion!




**Bridge electrode**

Please connect the remaining black electrode cable to the bridge electrode. This electrode has to be placed to the forehead by using the rubber band.

(Figure 13) Shows basic Connection montage of the Bipolar occipital Straddle to the amplifier. All 4 channels are referenced to one electrode connected to the Fz point. The 4 testing electrodes were connected to 4 amplifier slots numbered T1, T2, T3, and T4. The difference between each two testing pair would give rise to one channel, as shown in the channel configuration illustrated in figure 12. Each channel output was tested against a reference point Fz in a simple bipolar electrode recording. *Adapted from the Roland Consult manual.*

## 2.8.6 mfVEP Test parameters

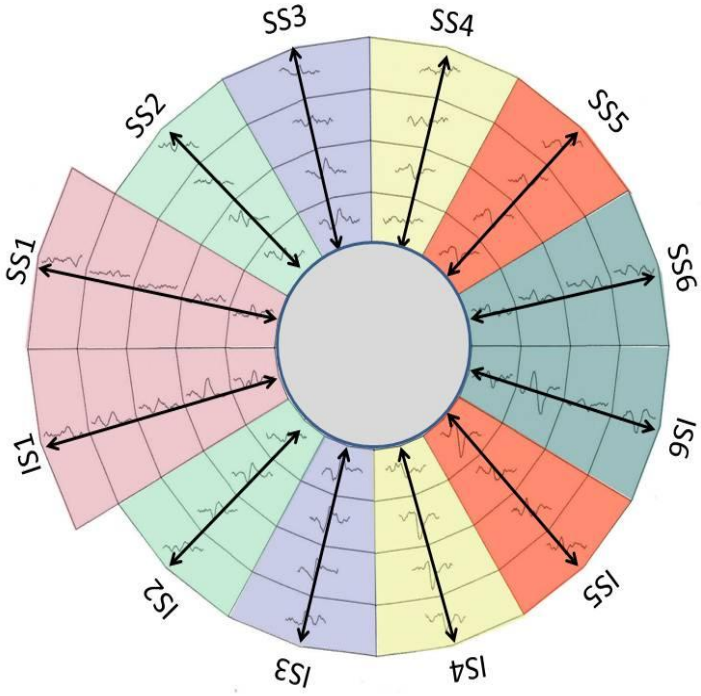
The main parameter in the hemifield sector analysis protocol of the mfVEP test is the SNR value. In order to measure the amplitude of these responses, the root mean square (RMS) was calculated for the standard signal between 0-500 milliseconds. RMS is an efficient method for signal analysis, and it is commonly used for its ease to interpret, it requires only the specification of a time interval to analyse the signal, instead of identifying a specific part of the response. In figure (1) a signal window between 0-200 milliseconds was used, and the noise window between 300-500 milliseconds. In addition to measuring the RMS of each of the 58 responses for each eye, a SNR measure was obtained as previously described. In order to obtain the SNR of any segment, a signal window (0-200 milliseconds) and a "noise-only" window (300-500 milliseconds) are specified. The SNR of any given response from an individual segment is obtained by using the following formula.

$$SNR = \frac{RMS\ signal}{Average\ RMS\ noise\ from\ 58\ sectors}$$

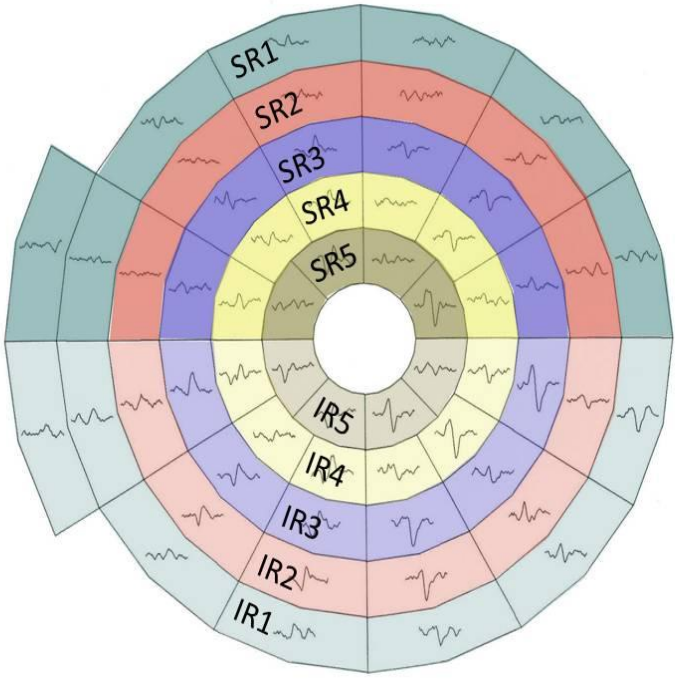
The average RMS noise is calculated by the average of the 58 segments X 4 channels = 232 RMS values of the noise-only window. The use of this method to calculate the SNR value will add the benefit of normalizing signals and make the responses closely variable in their noise levels from different segments. If any segment shows no response (no true signal) the SNR would have an average SNR equal to 1.0. The records from the 4 channels assure the acquisition of the best available signal from each location, by selecting the best SNR recorded and least noisy ones. All SNR records below 1.0 were not taken into analysis as these are pure noise with no trace of any signals.

## 2.8.7 The Hemifield Sector / Hemi-ring analysis (HSA) protocol

The concept of the analysis protocol as described earlier is to compare and divide the mfVEP field into similar (equal) sectors and hemi-rings across the horizontal meridian, where a sector at the superior hemisphere compared to its corresponding fellow sector in the inferior hemisphere. The allocated sectors, shown in (figure 14), comprise a fixed number of segments. After the SNR value from each segment is recorded, an average is taken from all the segments allocated to any given sector, to give a sector average SNR value. This averaged sector SNR value is the one that will be compared to its corresponding sector across the horizontal meridian, and not the individual segments. Similarly, the same process was completed for the hemi-rings, shown in (figure 15).




(Figure 14) Shows the allocated 6 sectors and its corresponding fellows in both hemispheres.
SS = superior sector, IS = inferior sector




(Figure 15) shows the 5 allocated hemi-rings and its corresponding fellows in both hemispheres.
SR = superior hemi-ring, IR = inferior hemi-ring

Certain measurable parameters were recorded and calculated in this HSA protocol in order to compare the 3 groups. The SNR value in each sector / hemi-ring was compared to the corresponding fellow in the opposite hemisphere and the difference between were calculated to check for any significant change between corresponding sectors / hemi- rings. The same SNR values and difference between sectors / hemi-rings were also compared to their similar identical ones in the 3 groups to check for any significant change between groups

## 2.9 HUMPHREY FIELD ANALYZER (HFA)

All study subjects had one or two HFA tests; the first to familiarize the test taker with the test process and to minimize the reliability errors. If the subject has already taken the test before and familiar with the procedure then this first test was waived. The second test was the study test and all its data was recorded and analysed if subjects were within the reliability criteria of 33% false positive and false negative rates and 20% fixation losses. All subjects underwent the SITA (Swedish Interactive Threshold Algorithms) Standard 24-2 automated perimetry using the Humphrey Field Analyser (HFA). The measurable parameters recorded in HFA test included the Mean Deviation (MD), the Pattern Standard Deviation (PSD) and the Glaucoma Hemifield Test grading (GHT). Most of these parameters were used in the HFA grading scale of visual field loss severity.

### 2.9.1 Visual field grading scale

Many grading scales of glaucomatous visual field loss were created to help clinicians grading the severity of glaucoma progression and use this in their management plan. The HAP (Hodapp-Anderson-Parrish) scale is one of the most commonly used in clinical practice [(216,217)]. It is a categorical scale that considers in its grading the size of the glaucomatous defect, depth of the defect, and proximity of the defect to fixation. The outcome of the grading scale can be utilized to classify the severity of glaucoma and to create intraocular pressure target plans. Their classification scale used two main criteria. The first criterion is the overall extent of the defect, which can be examined by assessing the mean deviation (MD) and the number of significant defective points in the Statpac-2 package of HFA system, derives from the total deviation probability plot. The second criterion is based on how close the defect is to the fixation point and how much of it has encroached to affect the central retinal sensitivity [(44,218)]. The minimum criteria for diagnosing glaucomatous damage and the criteria used to divide defect severity in three classes are presented in Table (1)

**Table (1) The HFA grading system used in this study**

| | |
|---|---|
| **Grade 1#** | Normal visual field |
| **Grade 2 Minimal visual field defect*** | 3 or more adjacent points in an expected location of the central 24° field that have p < 5% on the Pattern Standard Deviation (PSD)<br>Glaucoma Hemifield Test (GHT) "outside normal limits)<br>Corrected Pattern Standard deviation (CSPD) with p < 5% |
| **Grade 3 early visual field defect**** | Mean Deviation (MD) no worse than -6 dB<br>Pattern Standard Deviation (PSD) plot <25% of test points depressed below 5% level and <15% of test points depressed below the 1% level<br>No test point within central 5° |
| **Grade 4 moderate visual field defect**** | (MD) worse than -6 dB but not worse than -12 dB<br>(PSD) plot <50% of test points depressed below the 5% level and <25% of test points depressed below the 1% level<br>No test point within central 5°<br>Only one Hemifield containing a test point with sensitivity <15 dB within the central 5° of fixation |
| **Grade 5 severe visual field defect**** | (MD) worse than -12 dB<br>PSD plot >50% of test points depressed below the 5% level or >25% of test points depressed below the 1% level<br>Any test point within the central 5° with sensitivity ≤0dB<br>Both Hemifields containing test point(s) with sensitivity <15 dB within the central 5° of fixation |

#Normal field with no significant field changes
*From the Anderson DR criteria for minimal visual field defect [(219)]
** From the Hodapp-Anderson-Parrish grading scale [(216)]

## 2.9.2 Comparison of HFA and mfVEP tests

It is important to mention that care must be exercised when comparing HFA visual field with mfVEP test results because the displays for the two techniques, and the ways in which the fields are sampled, are very different [(220)]. Since the mfVEP display is scaled, the 58 regions are unequally sampled by the 24-2 HFA test locations. Practically speaking, there is no single method for combining HFA values (in decibels) from different field locations that can be considered a standard in this regard [(220,221)]. However, many previous studies have shown good agreement between the two tests using probability plots grading scores, but not through direct comparison of test parameters [(39,134,142,159,170,171,179-181)]. In this study, the most practical way of comparison was to compare two grading systems, instead of going through decibel conversion or matching testing degrees. A five division grading scale was created for the SNR values, ranging between 1 and 5,

where G1 is the best SNR value (>3), G2 = SNR values between 2.5-2.99, G3 = SNR values between 2-2.49, G4 = SNR values between 1.5-1.99 and G5 = SNR values between 1-1.49. All SNR values below 1 (<1) were considered pure noise, and were not used in the data analysis. These grades were compared to the HFA grading system of Hodapp, Parish and Anderson. The SNR grading scale is similar to the HFA grading system; the higher the grade the lower the SNR value, and the poorer the response. Thus an SNR value of 1, which means grade 5, is the poorest response could be obtained as opposed to SNR value of >3, which is grade 1, where the response is the highest and almost always reflects normal range.

# 3.0 RESULTS

## 3.1 DEMOGRAPHIC DATA OF STUDY POPULATION

### 3.1.1 Visual field and mfVEP examination

A total of 60 subjects (112 eyes) were recruited and enrolled in this study. All subjects finished the study tests effectively with no withdrwal during the study period. Overall performance and comprehension of the test procedure was good and accepted; almost all subjects (56/60) needed the routine instructions to get them started, while a few subjects (4/60) needed more detailed explanation and the test was restarted a few times. All subjects had two HFA - SITA standard visual tests and a single mfVEP test. All subjects, including experienced test-takers such as glaucoma patients, agreed to take the HFA test twice. All study subjects were able to finish 2 cycles of the mfVEP test successfully with no signifcant complaints or side effects. It was noted that only 3/112 eyes required a break during one of the test cycles upon her/his request. The majority of subjects (41/60) reported the mfVEP test as the easier test-taking process that did not need from their side, influential decisions. The rest (19/60) reported that HFA was easier and less stressful. It was also noted that most of glaucoma and glaucoma suspect patients (29/40) favoured the HFA test as a simple routine method for visual field check, while the rest (11/40) found the mfVEP better for variable reasons; simpler, advanced technology, easier and less confusing. The test duration for a SITA standard HFA test ranged between 4-11 minutes per eye, while the duration of a single (2 cycles) mfVEP tests ranged between 16-22 mintues per eye excluding preparation time. The mfVEP preparation time was similar in all subjects, ranging between 13-20 minutes per patient, which includes the setting for both eyes.

### 3.1.2 Study subjects

In order to eleminate the influence of age and gender on data, all subjects of the normal (control) group were carefully selected to match the two study groups, as possible, in both age and gender distribution. Table (2) shows that there were 20 normal subjects in the control group, a total of 38 eyes were tested. The glaucoma suspect group had 20 patients with a total of 38 eyes completing the tests. 20 glaucoma patients (36 eyes) were tested in the glaucoma group. There was no signficant age distribution difference detected among the 3 study groups ($P = 0.673$). The mean age for all study subjects were 40.65 years, with no signficiant preference between genders in any study group. There was no substantial variability in the age or gender among study groups, with a very close age range. The age ranged between 19-72 in all groups, the design of this study tried to cover a wide range of age matched responses in the 3 groups.

**Table (2) Analysis of age in all study groups**

| Groups | Gender | Number | | Age | | | Sig. |
|---|---|---|---|---|---|---|---|
| | | Subjects (%) | Eyes (%) | Mean | SD | Range of Age | |
| **All subjects** | Male | 31 (51) | 58 (52) | 39.51 | 12.521 | 19-68 | |
| | Female | 29 (49) | 54 (48) | 41.87 | 13.323 | 19-72 | |
| | Total | 60 (100) | 112 (100) | 40.65 | 12.910 | 19-72 | |
| **Normal** | Male | 10 (50) | 18 (47) | 39.15 | 14.412 | 19-66 | |
| | Female | 10 (50) | 20 (53) | 39.31 | 11.078 | 19-62 | |
| | Total | 20 (100) | 38 (100) | 39.24 | 12.685 | 19-66 | P Value* |
| **Glaucoma Suspect** | Male | 11 (55) | 20 (52) | 39.1 | 13.822 | 19-67 | 0.673 |
| | Female | 9 (45) | 18 (48) | 44.94 | 13.493 | 19-64 | |
| | Total | 20 (100) | 38 (100) | 41.87 | 13.808 | 19-67 | |
| **Glaucoma** | Male | 10 (50) | 19 (52) | 40.31 | 11.24 | 20-62 | |
| | Female | 10 (50) | 17 (48) | 41.47 | 10.65 | 21-63 | |
| | Total | 20 (100) | 36 (100) | 40.86 | 12.91 | 20-63 | |

*One way ANOVA test. Significant when $P < 0.05$

Table (2) shows the age distribution of all subjects in the 3 study groups. There was no signifcant statistical difference in age among groups that could have affected the data ($P = 0.964$ for normal group, 0.964 for glaucoma suspect group, and 0.810 for glaucoma group). This indicates that there was no signifcant influence of the age factor upon the data.

### 3.1.3 Normality tests

All SNR values from individual sectors / hemi-rings, in all groups, were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. It was found that almost all p values were >0.05, which indicates that the data followed a normal distribution. Normality was also checked using a Q-Q plot, for the purpose of graphical presentation which confirmed visually the same findings and the normal distribution of the SNR data. Sample plots from random sectors are shown in figure (16) for illustration.

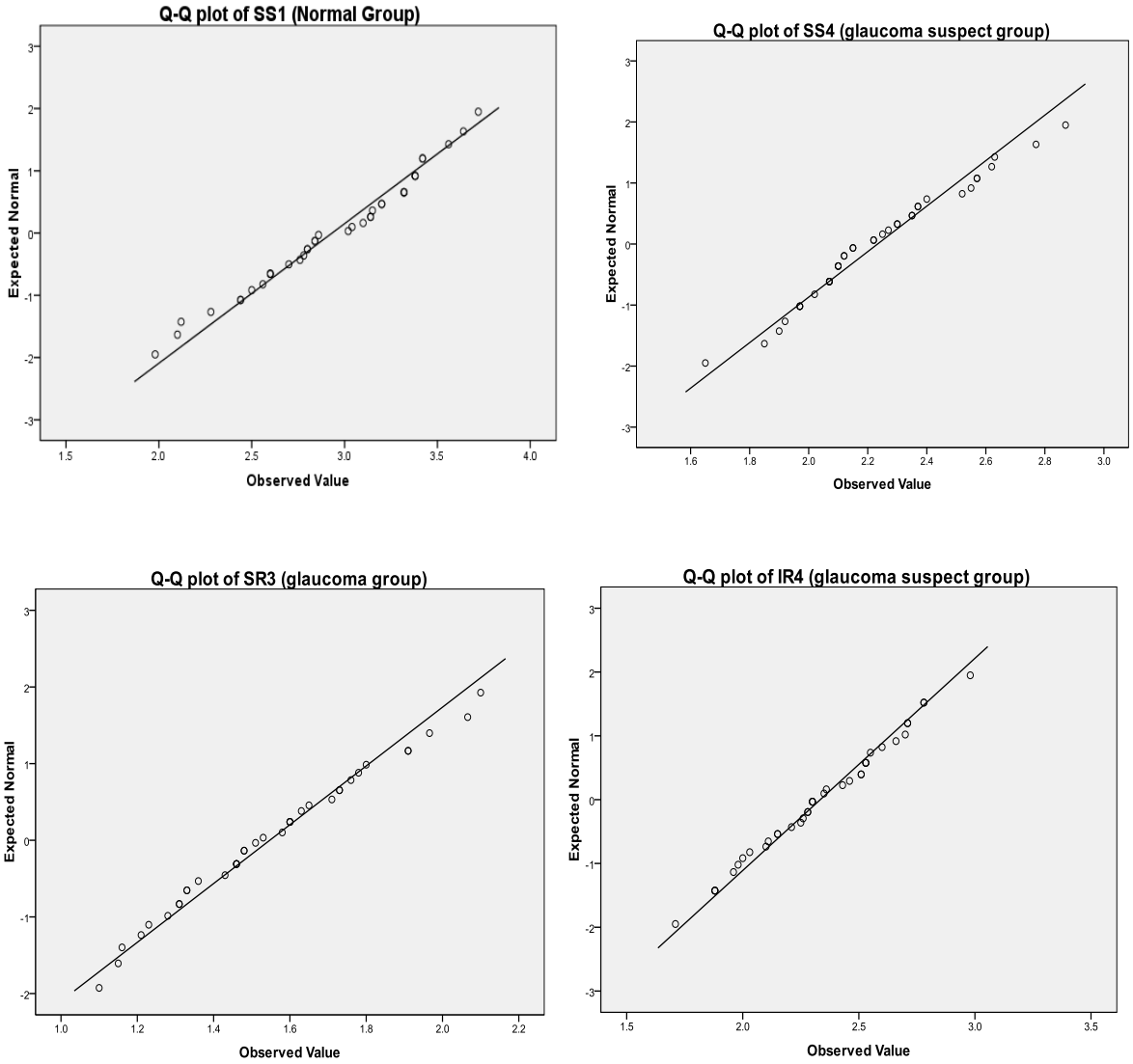



Figure (16) Q-Q plots for SNR values in random sectors / hemi-rings in the 3 study groups. The plots show accepted level of normal distribution

## 3.2 HUMPHREY VISUAL FIELD RESULTS

Two SITA standard 24-2 tests were performed for each tested eye. The test with the better response and least reliability errors was used for analysis. Many subjects were considered as well-trained on HFA test taking (36/60) most of which were glaucoma and glaucoma suspect patients who were used to undertaking the test on a regular basis.

### 3.2.1 Reliability of the tests

Most of the reported tests were counted as reliable (103/112) after looking at the reliability index values and the Glaucoma Hemifield Test (GHT) results. The rest of tested eyes (9/112) showed different forms of poor reliability on the two separate test occasions. Fixation Losses (FL) were the most common cause of poor reliability (5/9) followed by the False Negative (FN) error (3/9), and the False Positive (FP) erors were the least (1/9). Normal subjects yielded the majority of the reliable VF tests (36/38), followed by glaucoma patients (33/36) and the glaucoma suspect patients (34/38). Among the unreliable field group, glaucoma suspect patients were the majority (4/9), while normal subjects were the least unreliable among all study groups (2/9).

### 3.2.2 Global indices

Two global indices were recorded and utilized in the HFA grading system; the mean deviation (MD) and the pattern standard deviation (PSD). Table (3) shows the distribution of these parameters among all study groups

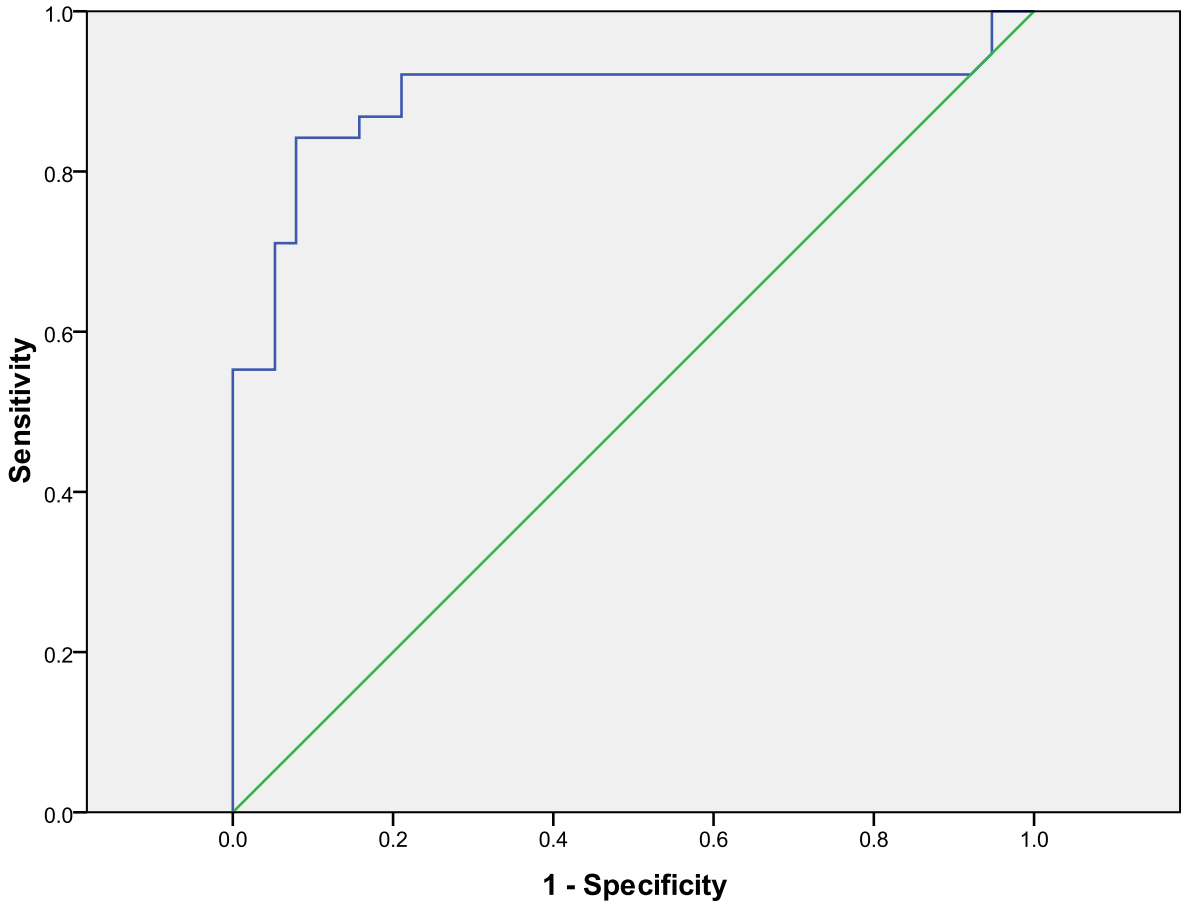
**Table (3) SITA standard global indeces of the 3 study groups**

| Index | Group | | | | | |
|---|---|---|---|---|---|---|
| | Normal | | GL Suspect | | Glaucoma | |
| | M | SD | M | SD | M | SD |
| Mean Deviation (MD) | 1.08 | 1.876 | -2.51 | 4.251 | -7.16 | 8.156 |
| Pattern Standard Deviation (PSD) | 1.73 | 0.571 | 2.64 | 2.484 | 4.57 | 3.093 |

Table (3) shows the values of mean deviation and pattern standard deviation of the 3 groups. There was evident higher values in the glaucoma and glaucoma suspect groups compared to normal group

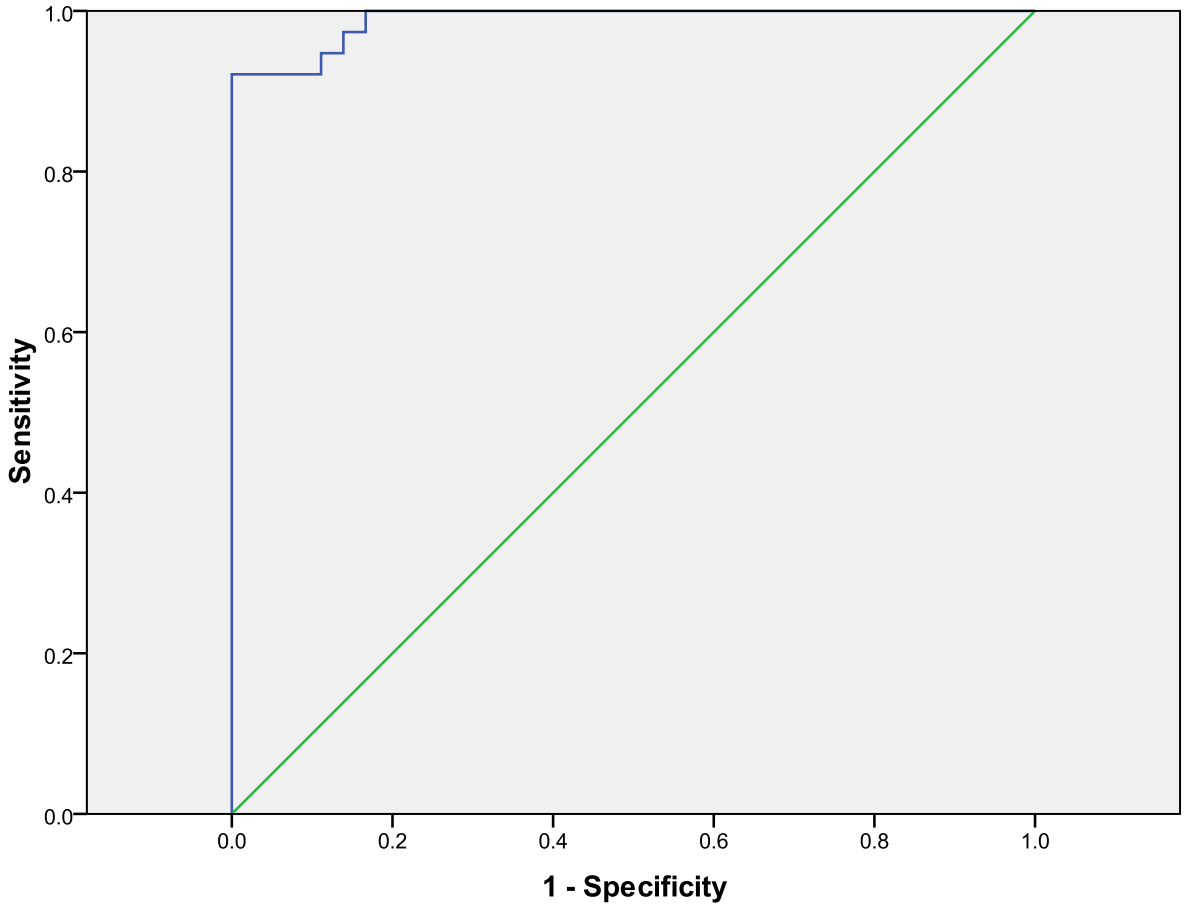
## 3.3 MFVEP SENSITIVITY AND SPECIFICITY FIGURES

### 3.3.1 ROC analysis for the glaucoma suspect group




(Figure 17) Area under curve = 0.892 Sensitivity 89% and specificity 79% for detection of glaucoma suspect. We calculated the 89% at 2.38 SNR value as the cut-off for glaucoma suspect.

### 3.3.2 ROC analysis for the glaucoma group




(Figure 18) Area under curve = 0.989. Sensitivity 97% and Specificity 86% for detection of glaucoma. We calculated the 97% at 1.99 SNR value as the cut-off for glaucoma.

### 3.3.3 Agreement between the HFA and mfVEP results

The ROC cut-off values for glaucoma and normal groups were used to perform Kappa statistical agreement tests. SNR value of 1.99 at a sensitivity level of 97% and specificity 0f 86% as detected by the ROC curve, was used as a cut-off value; where any response above this value were considered as normal response. Kappa statistical analysis showed a good agreement between HFA and SNR. There was 88.9% agreement between the two tests in identifying normal subjects, and 77.8% agreement in identifying glaucoma subjects. The agreement is higher and more appropriate for the identification of normal subjects, while slightly worse for identifying glaucoma subjects.

## 3.4 VISUAL FIELD GRADING SYSTEM

### 3.4.1 HFA grading analysis

The SITA visual field tests were graded according to Hoddap – Anderson criteria mentioned in the methods section. There are 5 grades identifyng the severity of the glaucomatous visual field loss. Table (4) shows the distribution of visual field test grades in all study groups. The Chi-square test was used to identify statistically signicant differences in the number of tests that were recorded in each grade between groups. There was a highly signifcant variation in the number of tests recorded in each group, indicating good validity of the grading system used. Grade 1 which means a normal visual field test with no abnormalities was recorded in (24/39) tests, while glaucoma suspects were (14/39) and only one test were classifed as having a normal field while the patient is known to have glaucoma. Conversly, grade 5 which indicates severe glaucamtous field loss was not recorded in any of the normal subjects' tests (0/16), while the majority were in the glaucoma groups (12/16) and glaucoma suspects were (4/16).

**Table (4) HFA grades among groups**

| HFA Grade | | Group | | | Total | Sig. |
|---|---|---|---|---|---|---|
| | | Normal | GL Suspect | Glaucoma | | |
| Grade | 1 | 24 | 14 | 1 | 39 | P value* **<0.001** |
| | 2 | 8 | 14 | 2 | 24 | |
| | 3 | 6 | 3 | 7 | 16 | |
| | 4 | 0 | 3 | 14 | 17 | |
| | 5 | 0 | 4 | 12 | 16 | |
| | Total | 38 | 38 | 36 | 112 | |

*Chi-square test. Significant when P < 0.05.

The glaucoma suspect group showed poor homogeneity in the clinical findings and reasons for suspected glaucoma. Whilst most of patients had one or more clinical findings suggestive of glaucoma, the majority did not have any visual field defects. However, 4 glaucoma suspect patients showed significant visual field defects which could have been considered as early glaucomatous defects rather than suspects.

### 3.4.2 SNR grading analysis

A grading system for the SNR values recorded by the mfVEP was devised. The system is simple and only involves 5 different ranges of SNR values as described earlier in the methods section. The average SNR for each sector and hemi-rings was calculated. The average SNR values from all sectors and hemi-rings for each field were graded according to the grading range shown in Table (5). Grade 1 stands for a healthy normal response where the signal is 3 times or more the average noise from the entire 58 field segments. Conversely, grade 5 stands for low SNR value where the signal is almost equal or slightly more than the average noise, which is a value we expect to be found in a damaged sector or a focal field defect. All SNR values <1 were considered as pure noise and were not counted in the analysis.

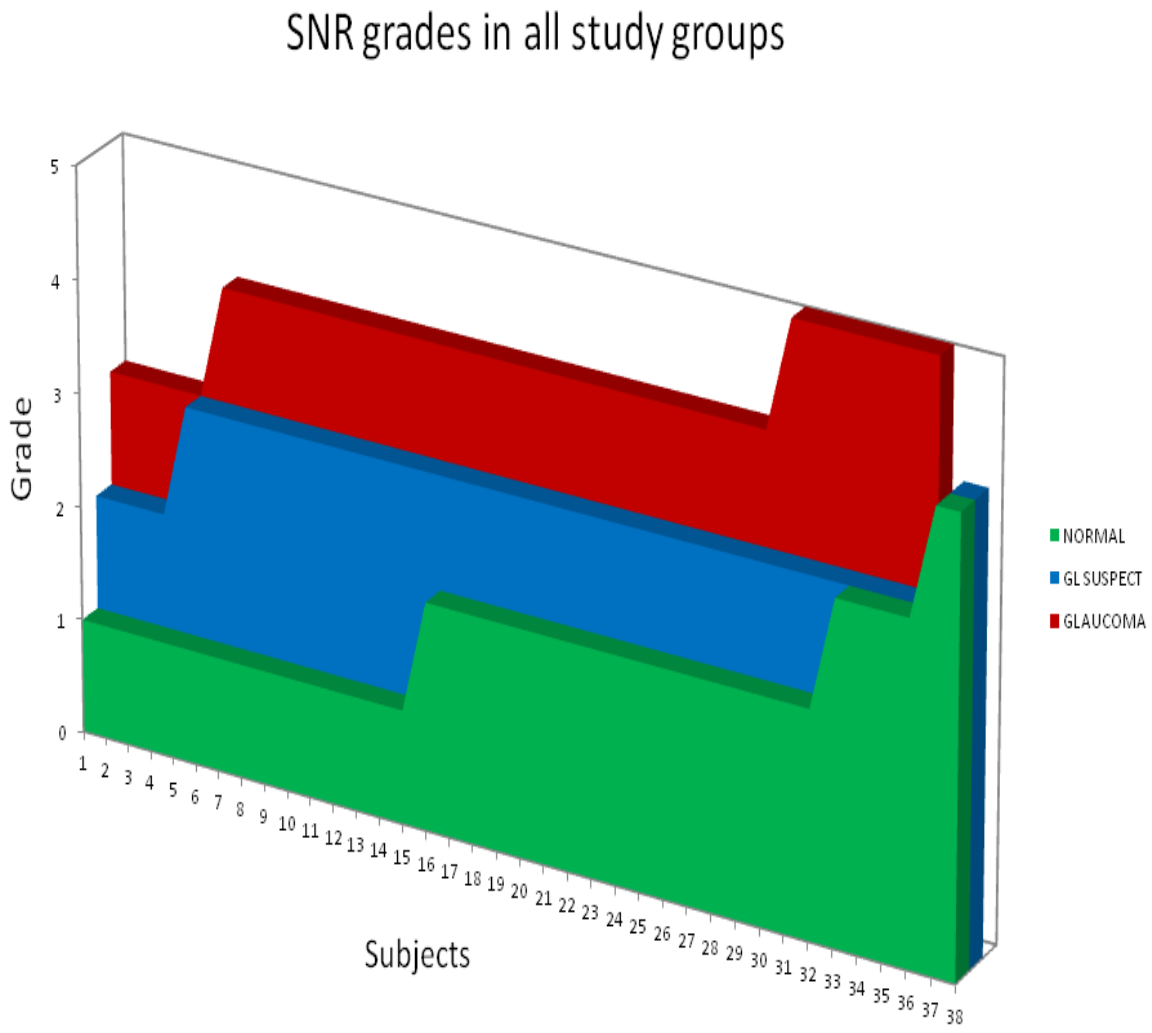
**Table (5) SNR Grades among groups**

| Grade (Average SNR Range) | Group | | | Total | Sig. |
|---|---|---|---|---|---|
| | Normal | GL suspect | Glaucoma | | |
| G1(≥3) | 15 | 0 | 0 | 15 | |
| G2 (2.5-2.99) | 17 | 4 | 0 | 21 | |
| G3 (2-2.49) | 4 | 32 | 5 | 41 | P value* |
| G4 (1.5-1.99) | 2 | 2 | 24 | 28 | **<0.001** |
| G5 (1-1.49) | 0 | 0 | 7 | 7 | |
| Total | 38 | 38 | 36 | 112 | |

*Chi-square test. Significant when P < 0.05

However, on the segment level, there were few <1 recordings mostly in patients with advanced glaucoma. On the sector / hemi-ring level, there was no <1 SNR value calculated because all <1.0 values were excluded from averaging each sector and hemi-ring. Interestingly, the table shows that grade 1 included 15 eyes; all of them were in the normal group, which gives a good credibility to the classification system. Similarly, grade 5 which is a poor response included 7 eyes; all of them

were glaucoma patients, which add more credibility to this classification. Using the Chi-square test we found a highly significant distribution of these groups among groups.




(Figure 19) shows the 3 study groups graded by the SNR grading system. The glaucoma suspect group (blue) was very well confined to the 2 middle grades (G2 and G3). There was an especially noticeable grade variation within the normal subject group. More consistently, grades in the glaucoma group were confined to poorer responses (G3-G5)

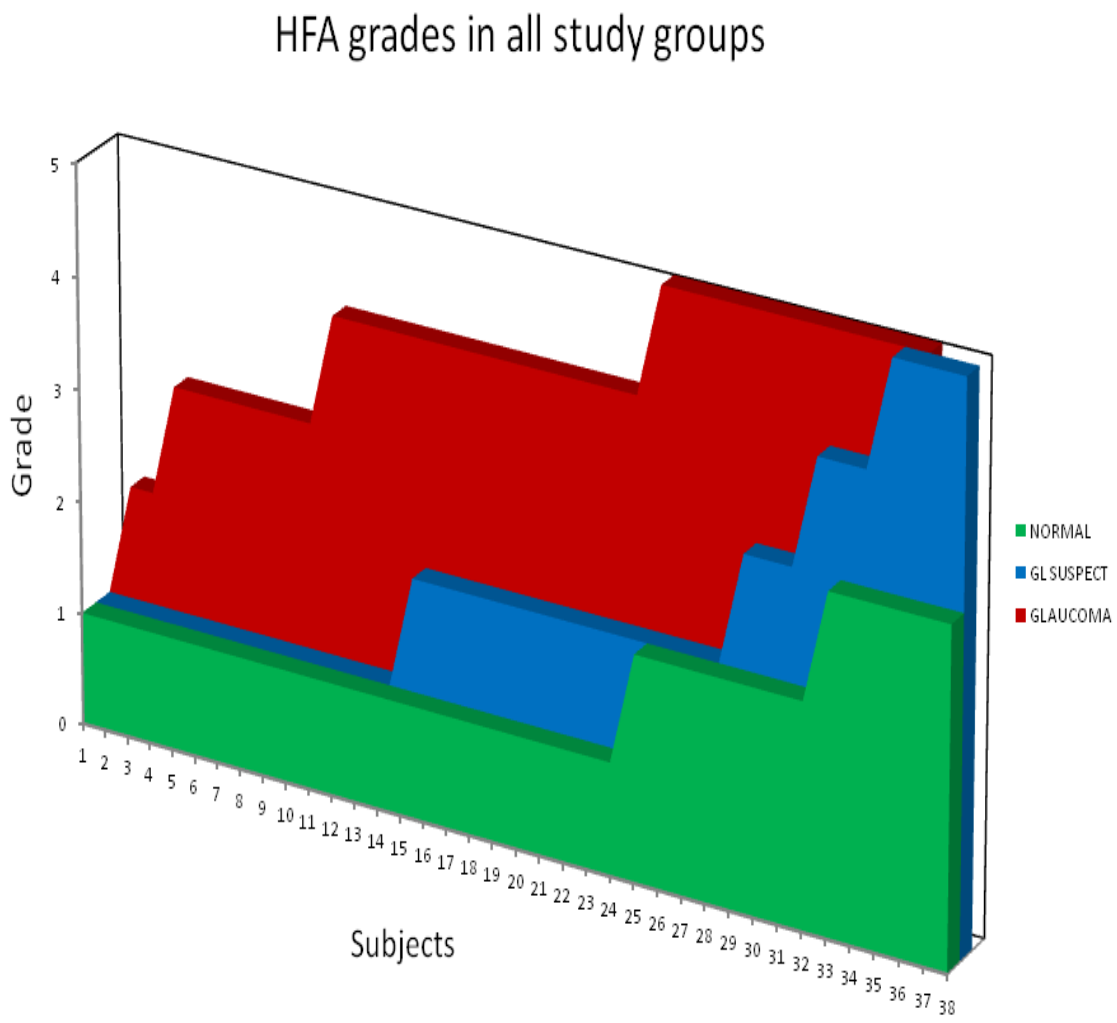



Figure (20) shows the 3 study groups graded by the HFA grading system. The glaucoma suspect group (blue) shows high variability; grades ranged between G1 and G5. There is also high variability seen in the glaucoma group which could be accepted as a natural presentation of the disease with variable levels of field damage. The HFA grading within the normal subject group was very consistent and confined to G1 and G2.

## 3.5 mfVEP TEST RESULTS

Two main parameters were tested in the 3 study groups; the SNR values in all sectors and hemi-rings, and the intersector / hemi-rings SNR difference values between each sector/hemi-ring and its corresponding fellow in the opposite hemifield. By using the two main parameters, 3 different types of comparisons were evaluated, within and between groups. Firstly, the SNR values were comapred in similar sectors / hemi-rings in all study groups in order to identify if there is any significant differences in SNR values in similar sectors / hemi-rings among groups. Initially, it was hypothesised that there should be a diminshed response and weaker signals in glaucoma patients compared to the normal control responses. Secondly, the intersector / hemi-rings SNR difference analysis between groups were recorded and analysed. According to the specified sectors and hemi-rings described in figure 14 & 15, the difference in SNR values between each sector / hemi-ring and its corresponding fellow in the opposite hemifield were calculated. These differences were compared then to its similar ones in all study groups in order to identify any significance. The hypothesis was that normal controls should not exhibit a significant difference between superior and inferior hemifield recordings, whether that was calculated in clusters of sectors or rings, because of the expected homogenity of responses in normal subjects, even if there is topographical variability in the field recoridngs accroding to location and eccentricity, but the similarity between the two healthy hemifields should not be affected. Conversly, and based on the glaucoma pathophysiological process, glaucomatous field defects should show some level of statistically significant difference between corresponding sectors and rings across midline, which should be reflected as a signifcant difference between the two hemifield when compared to the normal controls. Thirdly, the intersector / hemi-rings differences were compared as an independent measurable parameter between the 3 groups. We expected that the calculated differences should show some signifcance between groups.

### 3.5.1 Intra-group analysis

The SNR value was recorded for each segment of the entire 58 segments of the visual field. The field was automatically divided into sectors and hemi-rings according to the map shown in figure 14 & 15. The SNR for any given sector or hemi-ring is the average SNR from all the segments lie within the sector or hemi-ring. The SNR difference between corresponding sectors and hemi-rings in both hemifields were calculated. Both SNR values and SNR difference values were analysed and tested for signifcance within the same group, comparing sectors and hemi-rings within the same eye. Inter-eye asymmetry analysis was not carried out; all eyes were counted and tested independantly as separate entities. Only the Hemifield Sector Analysis protocol was applied.

### 3.5.1.1 The Normal group

20 normal subjects (38 eyes) were recruited and tested. All subjects showed during assessment and examination no history or clinical findings of ocular diseases that could interfere with the field of vision as listed in the selection criteria. Most of normal subjects (18/20) were included to test both eyes, only two subjects preferred to be included for single eye test. The inter-sector/hemi-ring differences between corresponding hemifields were tested for significance. 6 sectors and 5 hemi-rings on each hemifield were compared to their corresponding fellows on the opposite hemifield. A paired t-test was performed to check for statistical significance for all sectors and hemi-rings. Table (6) shows the results in details; there was only one sector (1/6) found signifcant when compared to its fellow one on the opposite hemifield, and no hemi-rings (0/5) were found to be signifcant. Figure (21) demonstrates the findings clearly in a graphical presentation. The mean SNR for normal group was 2.85 ± 0.503, which is a representative value for average normal responses.

### 3.5.1.2 The glaucoma suspect group

20 glaucoma suspect patients (38 eyes) were tested. The majority of patients (9/20) were diagnosed as glaucoma suspect based on high intraocular pressure (mean IOP 24 ± 3.75) recorded on multiple occasions, (5/20) had suspicious visual field results, (3/20) had suspicious optic disc appearance without established damage. Subjects in these groups were very familiar with the standard HFA test, however they wanted to get a notification of the mfVEP results once ready, hoping that the test would offer more conclusive answers about their condition. The inter-sector/hemi-ring differences between corresponding hemifields were tested for significance. 6 sectors and 5 hemi-rings on each hemifield were compared to their corresponding fellows on the opposite hemifield. A paired t-test was performed to check for statistical significance for all sectors and hemi-rings. Table (7) shows that 4/6 sectors were found to have statistically signifcant SNR difference when compared to their corresponding fellows, while 1/5 hemi-rings was statistically significant. Figure (22) demonstrates these findings in a graphical presentation. The mean SNR value for glaucoma suspect group was 2.27 ± 0.276, which is as expected, mildly lower than normal average but not considered as abnormal response.

**Table (6) Comparison between SNR values in superior and inferior hemifield sectors and hemi-rings in Normal group**

| Variables | Superior Hemifield | | | Inferior Hemifield | | | P – value* |
|---|---|---|---|---|---|---|---|
| | N | M | SD | N | M | SD | |
| Pair 1 (SS1-IS1) | 38 | 2.92 | 0.441 | 38 | 3.01 | 0.553 | 0.136 |
| Pair 2 (SS2-IS2) | 38 | 2.78 | 0.463 | 38 | 2.83 | 0.540 | 0.364 |
| Pair 3(SS3-IS3) | 38 | 2.86 | 0.520 | 38 | 2.80 | 0.545 | 0.228 |
| Pair 4 (SS4-IS4) | 38 | 2.77 | 0.485 | 38 | 2.85 | 0.479 | 0.106 |
| Pair 5 (SS5-IS5) | 38 | 2.89 | 0.536 | 38 | 2.75 | 0.521 | **0.028** |
| Pair 6 (SS6-IS6) | 38 | 2.84 | 0.550 | 38 | 2.93 | 0.490 | 0.298 |
| Pair 7 (SR1-IR1) | 38 | 2.84 | 0.462 | 38 | 2.88 | 0.505 | 0.321 |
| Pair 8 (SR2-IR2) | 38 | 2.82 | 0.538 | 38 | 2.86 | 0.538 | 0.478 |
| Pair 9 (SR3-IR3) | 38 | 2.95 | 0.559 | 38 | 2.87 | 0.469 | 0.148 |
| Pair 10 (SR4-IR4) | 38 | 2.81 | 0.415 | 38 | 2.87 | 0.566 | 0.320 |
| Pair 11 (SR5-IR5) | 38 | 2.85 | 0.456 | 38 | 2.76 | 0.455 | 0.091 |

(Table 6) shows that only one pair of sectors (SS5-IS5) has statistically significant difference in the SNR value. All other tested sectors and hemi-rings were not statistically significant.
*Paired t-test, significant when $p < 0.05$

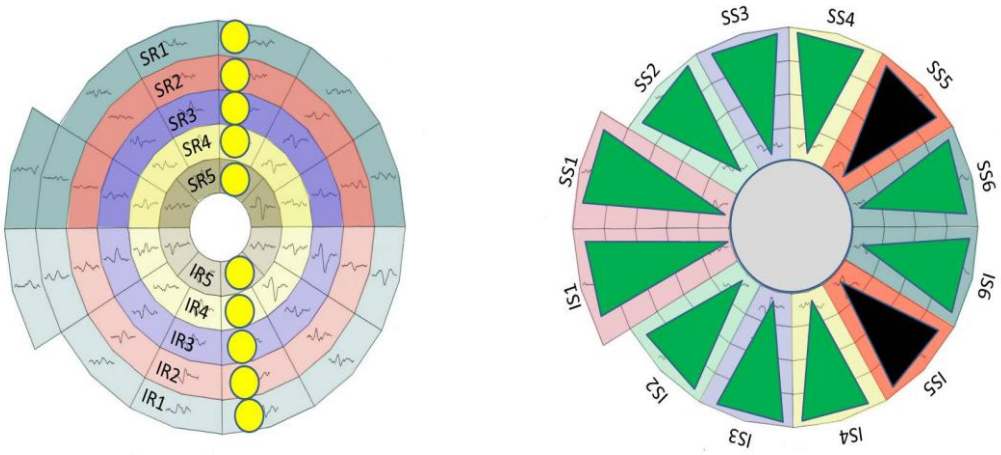



Figure (21) illustrates that only one pair of sectors (SS5-IS5) marked with (black triangle) was found to have statistically significant difference in its SNR value. All other sectors showed no statistical difference (green triangle). None of the hemi-rings showed a statistically significant difference between the two hemifields (yellow circles)

**Table (7) Comparison between SNR values in superior and inferior hemifield sectors and hemi-rings in Glaucoma Suspect group**

| Variables | Superior Hemifield | | | Inferior Hemifield | | | P – value* |
|---|---|---|---|---|---|---|---|
| | N | M | SD | N | M | SD | |
| Pair 1 (SS1-IS1) | 38 | 2.03 | 0.432 | 38 | 2.18 | 0.515 | 0.232 |
| Pair 2 (SS2-IS2) | 38 | 1.85 | 0.355 | 38 | 2.20 | 0.476 | **0.001** |
| Pair 3(SS3-IS3) | 38 | 1.74 | 0.251 | 38 | 2.28 | 0.596 | **<0.001** |
| Pair 4 (SS4-IS4) | 38 | 1.65 | 0.274 | 38 | 2.28 | 0.632 | **<0.001** |
| Pair 5 (SS5-IS5) | 38 | 1.84 | 0.385 | 38 | 2.14 | 0.462 | **0.003** |
| Pair 6 (SS6-IS6) | 38 | 1.98 | 0.506 | 38 | 2.15 | 0.493 | 0.69 |
| Pair 7 (SR1-IR1) | 38 | 2.17 | 0.212 | 38 | 2.29 | 0.295 | **0.010** |
| Pair 8 (SR2-IR2) | 38 | 2.24 | 0.295 | 38 | 2.22 | 0.218 | 0.556 |
| Pair 9 (SR3-IR3) | 38 | 2.24 | 0.233 | 38 | 2.29 | 0.299 | 0.225 |
| Pair 10 (SR4-IR4) | 38 | 2.33 | 0.232 | 38 | 2.33 | 0.306 | 0.930 |
| Pair 11 (SR5-IR5) | 38 | 2.34 | 0.315 | 38 | 2.31 | 0.295 | 0.508 |

(Table 7) shows that 4 pairs of sectors (4/6) have statistically significant difference in the SNR value, while only one hemi-ring (1/5) showed statistically significant difference. All other tested sectors and hemi-rings were not statistically significant.

*Paired t-test, significant when $p < 0.05$

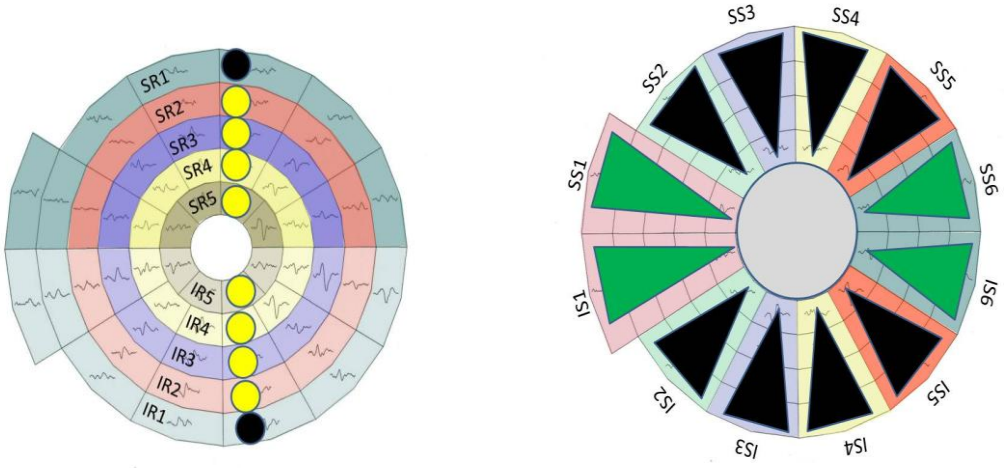



Figure (22) illustrates that 4 pairs of sectors marked with (black triangle) were found to have statistically significant difference in its SNR value. Only one hemi-ring pair was statistically significant (black circle) All other sectors showed no statistical difference (green triangle) and the rest of hemi-rings did not show any statistically significant difference between the two hemifields (yellow circles)

### 3.5.1.3 The glaucoma group

20 glaucoma patients (36 eyes) were tested. The majority of patients (14/20) had simple open angle glaucoma (OAG), (3/20) had narrow angle glaucoma (NAG), and (1/20) had pseudoexfoliation glaucoma. They ranged between “mild” and “severe” glaucomatous field losses using the Hoddap-Anderson criteria described earlier in the methods section. Most of the patients (13/20) had asymmetrical glaucomatous field changes between eyes, the rest (7/20) had symmterical mild to moderate field changes. Only 8/36 eyes showed advanced glaucomatous changes with symmterical severe field loss across midline in both hemifields, all the rest (28/36) showed clear differences between the two corresponding hemifields. The inter-sector/hemi-ring differences between corresponding hemifields were tested for significance. 6 sectors and 5 hemi-rings on each hemifield were compared to their corresponding fellows on the opposite hemifield. A paired t-test was performed to check for statistical significance for all sectors and hemi-rings. Table (8) shows that all sectors and hemi-rings were statistically signifcant when compared to their corresponding fellows in the opposite hemifield. Figure (23) demonstrates the findings graphically. The results show that the majority of patients had asymmetrical glaucomatous visual field defects between the two eyes, which is a good confirmation of how important the monocular mfVEP test analysis is. The monocular analysis is more beneficial in these patients simply because inter-eye analysis, which assumes that one eye is a “normal” reference for the glaucomaouts one, will not detect the defects accurately. The mean SNR value for glaucoma group was 1.70 ± 0.412, which is low average response close to poor (SNR = 1). This SNR average confirms the ability of the HSA protocol to identify glaucomatous visual field defects accurately.

**Table (8) Comparison between SNR values in superior and inferior hemifield sectors and rings in Glaucoma group**

| Variables | Superior Hemifield | | | Inferior Hemifield | | | P – value* |
|---|---|---|---|---|---|---|---|
| | N | M | SD | N | M | SD | |
| Pair 1 (SS1-IS1) | 36 | 1.54 | 0.222 | 36 | 1.74 | 0.376 | **0.001** |
| Pair 2 (SS2-IS2) | 36 | 1.58 | 0.215 | 36 | 1.83 | 0.495 | **0.002** |
| Pair 3(SS3-IS3) | 36 | 1.46 | 0.313 | 36 | 1.82 | 0.456 | **<0.001** |
| Pair 4 (SS4-IS4) | 36 | 1.44 | 0.245 | 36 | 1.81 | 0.489 | **<0.001** |
| Pair 5 (SS5-IS5) | 36 | 1.58 | 0.286 | 36 | 1.88 | 0.438 | **<0.001** |
| Pair 6 (SS6-IS6) | 36 | 1.70 | 0.359 | 36 | 1.85 | 0.472 | **0.033** |
| Pair 7 (SR1-IR1) | 36 | 1.50 | 0.208 | 36 | 1.74 | 0.331 | **<0.001** |
| Pair 8 (SR2-IR2) | 36 | 1.52 | 0.265 | 36 | 1.70 | 0.413 | **0.002** |
| Pair 9 (SR3-IR3) | 36 | 1.54 | 0.264 | 36 | 1.90 | 0.495 | **<0.001** |
| Pair 10 (SR4-IR4) | 36 | 1.61 | 0.341 | 36 | 1.90 | 0.361 | **0.001** |
| Pair 11 (SR5-IR5) | 36 | 1.77 | 0.532 | 36 | 2.02 | 0.552 | **0.001** |

(Table 8) shows that all pairs of sectors (6/6) and hemi-rings (5/5) have statistically significant SNR difference

*Paired t-test, significant when $p < 0.05$

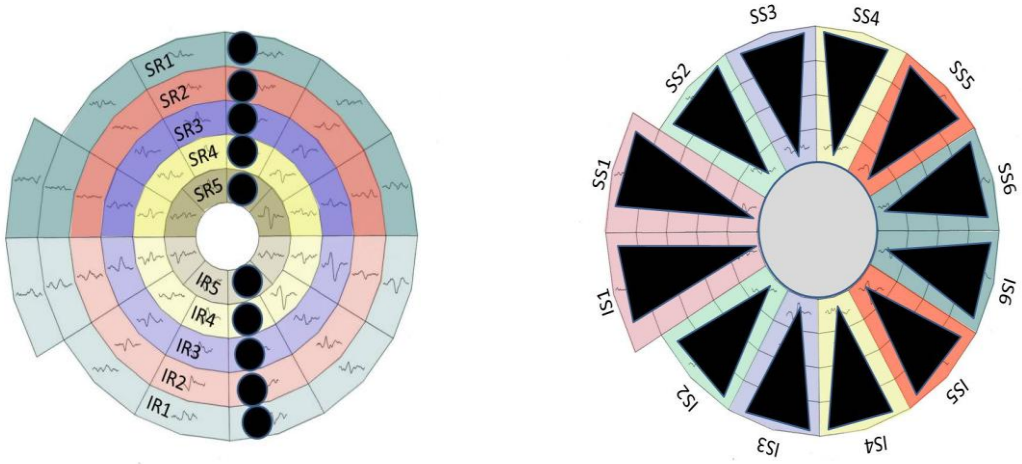



Fig (23) illustrates that all pairs of sectors marked with (black triangle), and pairs of hemi-rings marked with (black circles) were found to have statistically significant SNR difference.

## 3.5.2 Inter-group analysis

Two analysis procedures were used to compare the 3 groups; firstly, the average SNR value from each sector / hemi-ring was compared to its similar ones in the 3 groups to idenftify any statistically signifcant differences in the SNR responses. Secondly, the difference between each pair of corresponding sectors / hemi-rings within one group was compared to its similar difference values in the other groups. A one way ANOVA test was performed to check for statistical significance between groups. It is considered significant at 95% confidence interval when $P < 0.05$.

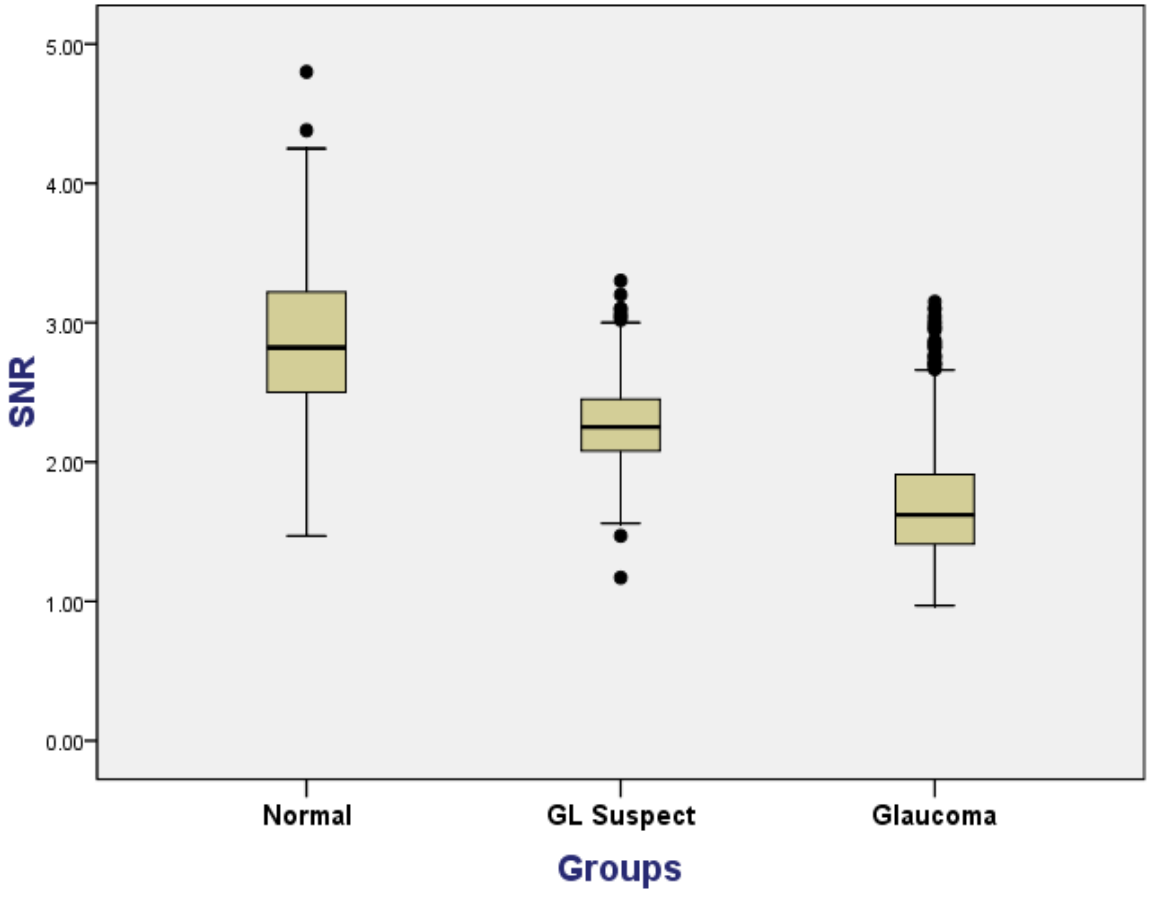
### 3.5.2.1 The SNR analysis

The SNR values of all sectors and hemi-rings were compared to its similar ones in the 3 study groups and was found to be statistically significant. Table (9) shows that the normal groups represented the highest mean (mean 2.85 ± 0.503) indicating better responses and signal strength, while the glaucoma groups showed the lowest mean (mean 1.7 ± 0.412) indicating poor responses and signals. The glacuoma suspect groups had a middle range of mean and lower variability (mean 2.27 ± 0.276). Table (10) shows the SNR values in each individual sector and hemi-ring compared to its similar ones in the 3 groups. It was expected that a lower response in the glaucomatous field sectors and hemi-rings would be found, thus the signal obtained and recorded from it should be signifcantly lower than the normal group. The glaucoma suspect sectors and hemi-rings also exhibited a significant difference when compared to the normal group. Figure (24) shows the box plot representation of the 3 groups. The clear difference between the SNR values in the 3 groups confirms the intital hypothesis and expectations that the mfVEP analysis protocol can differentiate between glaucomatous and normal visual fields. It can also identify by categorizing the SNR values, the early visual field changes, and present it as multiple focal depressions in SNR values when compared to normal responses.

**Table (9) Comparison of total SNR values in all sectors between study groups**

| Groups | N | M | SD | 95% Confidence Interval for Mean | | P – value* |
|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | |
| Normal | 836 | 2.85 | 0.503 | 2.823 | 2.892 | **<0.001** |
| Glaucoma Suspect | 836 | 2.27 | 0.276 | 2.252 | 2.290 | |
| Glaucoma | 792 | 1.70 | 0.412 | 1.677 | 1.734 | |
| Total | 2464 | 2.28 | 0.621 | 2.264 | 2.313 | |

*One way ANOVA test, significant when p < 0.05




(Figure 24) shows the box plot of mean SNR values in the 3 groups

**Table (10) Comparison of SNR values of sectors and hemi-rings between groups**

| Variables | Normal group | | | Glaucoma Suspect | | | Glaucoma | | | P – Value* |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | M | SD | N | M | SD | N | M | SD | |
| SS1 | 38 | 2.93 | 0.442 | 38 | 2.32 | 0.242 | 36 | 1.54 | 0.225 | **<0.001** |
| IS1 | 38 | 3.01 | 0.551 | 38 | 2.34 | 0.253 | 36 | 1.74 | 0.372 | **<0.001** |
| SS2 | 38 | 2.78 | 0.465 | 38 | 2.21 | 0.295 | 36 | 1.58 | 0.216 | **<0.001** |
| IS2 | 38 | 2.83 | 0.542 | 38 | 2.25 | 0.266 | 36 | 1.83 | 0.498 | **<0.001** |
| SS3 | 38 | 2.86 | 0.526 | 38 | 2.17 | 0.288 | 36 | 1.46 | 0.314 | **<0.001** |
| IS3 | 38 | 2.80 | 0.542 | 38 | 2.34 | 0.304 | 36 | 1.82 | 0.457 | **<0.001** |
| SS4 | 38 | 2.77 | 0.485 | 38 | 2.23 | 0.265 | 36 | 1.44 | 0.249 | **<0.001** |
| IS4 | 38 | 2.85 | 0.476 | 38 | 2.24 | 0.296 | 36 | 1.81 | 0.485 | **<0.001** |
| SS5 | 38 | 2.89 | 0.538 | 38 | 2.21 | 0.262 | 36 | 1.58 | 0.284 | **<0.001** |
| IS5 | 38 | 2.75 | 0.523 | 38 | 2.28 | 0.341 | 36 | 1.88 | 0.436 | **<0.001** |
| SS6 | 38 | 2.84 | 0.553 | 38 | 2.28 | 0.255 | 36 | 1.70 | 0.352 | **<0.001** |
| IS6 | 38 | 2.93 | 0.495 | 38 | 2.25 | 0.238 | 36 | 1.85 | 0.473 | **<0.001** |
| SR1 | 38 | 2.84 | 0.466 | 38 | 2.17 | 0.219 | 36 | 1.50 | 0.201 | **<0.001** |
| IR1 | 38 | 2.88 | 0.509 | 38 | 2.29 | 0.296 | 36 | 1.74 | 0.335 | **<0.001** |
| SR2 | 38 | 2.82 | 0.532 | 38 | 2.24 | 0.295 | 36 | 1.52 | 0.269 | **<0.001** |
| IR2 | 38 | 2.86 | 0.536 | 38 | 2.22 | 0.216 | 36 | 1.70 | 0.418 | **<0.001** |
| SR3 | 38 | 2.95 | 0.555 | 38 | 2.24 | 0.232 | 36 | 1.54 | 0.264 | **<0.001** |
| IR3 | 38 | 2.87 | 0.469 | 38 | 2.29 | 0.293 | 36 | 1.90 | 0.495 | **<0.001** |
| SR4 | 38 | 2.81 | 0.418 | 38 | 2.33 | 0.238 | 36 | 1.61 | 0.346 | **<0.001** |
| IR4 | 38 | 2.87 | 0.565 | 38 | 2.33 | 0.304 | 36 | 1.90 | 0.365 | **<0.001** |
| SR5 | 38 | 2.85 | 0.453 | 38 | 2.34 | 0.316 | 36 | 1.77 | 0.538 | **<0.001** |
| IR5 | 38 | 2.76 | 0.455 | 38 | 2.31 | 0.295 | 36 | 2.02 | 0.555 | **<0.001** |

(Table 10) shows a comparison of SNR values in each sector and hemi-ring between the 3 groups. There was a highly significant statistical difference ($p < 0.001$) between groups in all tested sectors and hemi-rings.

*One way ANOVA test, significant when $p < 0.05$

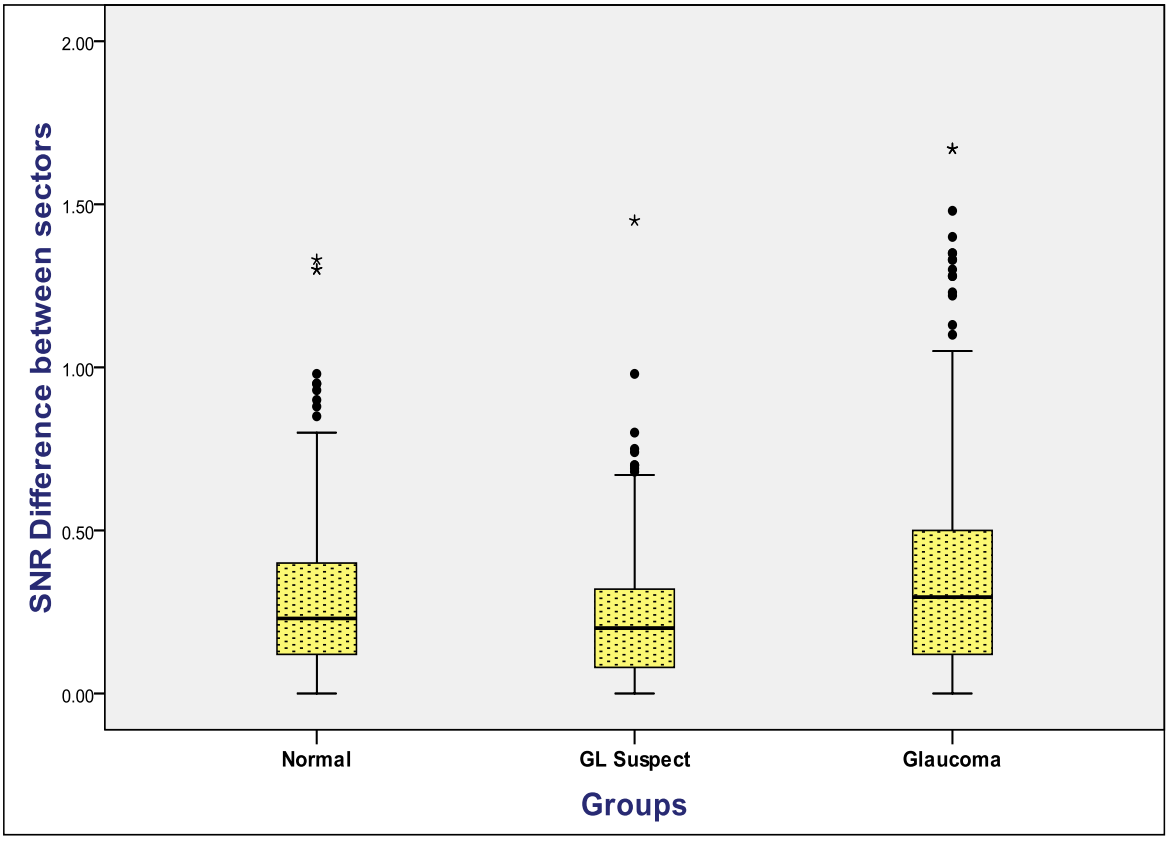
### 3.5.2.2 The SNR difference analysis

The SNR difference values between each pair of corresponding sectors and hemi-rings in each group were calculated and compared to its similar difference values in the other study groups in order to identify any statistical significance. The expectation was that the SNR difference vlaues between superior and inferior sectors and hemi-rings in glaucoma group would be significantly higher than the SNR difference values in the other two groups. Figure (25) shows a box plot presentation of the mean SNR difference values in the 3 groups. The range of SNR difference values in glaucoma group was higher and the mean was significantly higher than the normal group. Table (11) shows that the glaucoma group had the largest SNR difference value (mean 0.36 ± 0.321), compared to the normal (mean 0.28 ± 0.223) and glaucoma suspect group (mean 0.22 ± 0.184). This significant difference indicates as expected a higher intersector/hemi-ring SNR difference values across the midline compared to normal subjects and glaucoma suspect groups. Table (12) shows the SNR difference values calculated for each pair of corresponding sectors and hemi-rings in each group. 11 pairs of intersector/hemi-ring SNR difference values were compared within the 3 groups to identify any statistically significant difference among groups. This SNR difference values was statistically significant in 4 sectors and 3 hemi-rings. The rest of tested sectors and hemi-rings were not statistically significant (Figure 26).

**Table (11) Comparison of total intersector/hemi-ring SNR difference values between all study groups**

| Groups | N | M | SD | 95% Confidence Interval for Mean | | P – value * |
|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | |
| Normal | 418 | 0.28 | 0.223 | 0.260 | 0.303 | **<0.001** |
| Glaucoma Suspect | 418 | 0.22 | 0.184 | 0.209 | 0.244 | |
| Glaucoma | 396 | 0.36 | 0.321 | 0.335 | 0.398 | |
| Total | 1232 | 0.29 | 0.254 | 0.276 | 0.304 | |

(Table 11) shows the comparison between study groups of Intersector differences added up from all 11 sectors and rings. There is a highly significant difference between study groups when all sectors added up and compared collectively.
*One way ANOVA test. Significant if $p < 0.05$

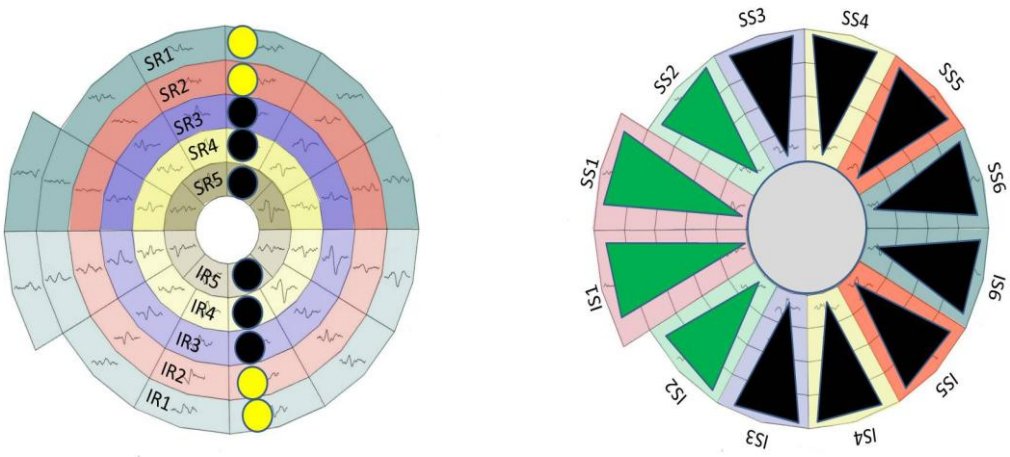



(Figure 25) shows a box plot of the mean of the 3 study groups

**Table (12) Comparison of individual hemifield Intersector/hemi-ring SNR Difference values**

| Variables | Normal group | | | Glaucoma Suspect | | | Glaucoma | | | P – Value * |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | M | SD | N | M | SD | N | M | SD | |
| SS1-IS1 | 38 | 0.27 | 0.201 | 38 | 0.22 | 0.195 | 36 | 0.29 | 0.241 | 0.289 |
| SS2-IS2 | 38 | 0.30 | 0.196 | 38 | 0.25 | 0.274 | 36 | 0.36 | 0.385 | 0.265 |
| SS3-IS3 | 38 | 0.27 | 0.189 | 38 | 0.28 | 0.231 | 36 | 0.48 | 0.480 | **0.004** |
| SS4-IS4 | 38 | 0.23 | 0.198 | 38 | 0.25 | 0.175 | 36 | 0.42 | 0.377 | **0.005** |
| SS5-IS5 | 38 | 0.33 | 0.205 | 38 | 0.24 | 0.185 | 36 | 0.38 | 0.289 | **0.045** |
| SS6-IS6 | 38 | 0.38 | 0.317 | 38 | 0.18 | 0.169 | 36 | 0.32 | 0.281 | **0.004** |
| SR1-IR1 | 38 | 0.21 | 0.126 | 38 | 0.24 | 0.153 | 36 | 0.28 | 0.170 | 0.355 |
| SR2-IR2 | 38 | 0.25 | 0.184 | 38 | 0.20 | 0.168 | 36 | 0.29 | 0.235 | 0.156 |
| SR3-IR3 | 38 | 0.26 | 0.268 | 38 | 0.22 | 0.165 | 36 | 0.44 | 0.355 | **0.002** |
| SR4-IR4 | 38 | 0.28 | 0.274 | 38 | 0.21 | 0.146 | 36 | 0.36 | 0.283 | **0.008** |
| S45-IR5 | 38 | 0.25 | 0.212 | 38 | 0.17 | 0.129 | 36 | 0.35 | 0.303 | **0.003** |

(Table 12) shows significant differences between groups in the Intersector comparison (superior and inferior). Most of the tested sectors were significant (7/11). (N) Number of eyes, (M) mean difference in SNR values, (SD) standard deviation.
*One way ANOVA test, significant when $p < 0.05$




(Figure 26) ilustartes that 4 pairs of sectors marked with (black triangle), and 3 pairs of hemi-rings marked with (black circles) were found to have a statistically significant SNR difference between groups. The rest of sectors (green traingle) and hemi-rings (yellow circles) were not statistically significant.

## 3.6 THE HEMIFIELD SECTOR ANALYSIS AND SITA STANDRD TEST RESULTS

### 3.6.1 Components of the Hemifield sector / hemi-ring analysis printout

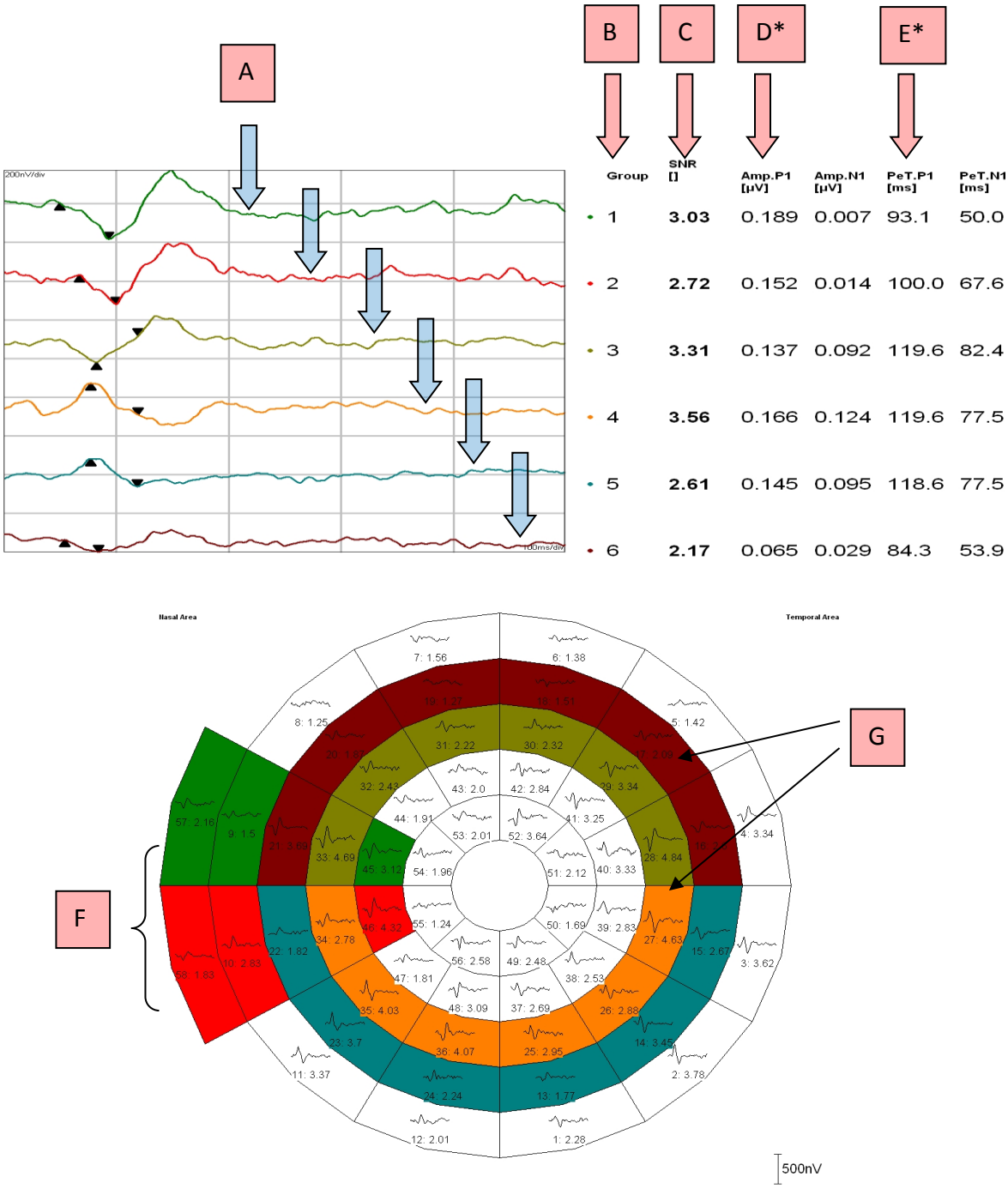



Figure (27) analysis components; colour-coded averaged waveforms from the sector or hemi-ring (A), sector / hemi-ring waveform numbers (B), averaged SNR value for each sector / hemi-ring (C), positive peak amplitude (D), positive peak latency (E), colour-coded sectors for comparison (F), colour-coded hemi-rings for comparison (G). *The amplitude markers (black arrow heads) are misplaced and should be ignored in this analysis because only SNR values were analysed without the use of amplitude values of positive waveforms or their latency. The beta version of the analysis software did not delete the amplitude category in the final analysis output, which will be modified and adjusted in future versions of the software.

## 3.6.2 Examples of recording errors

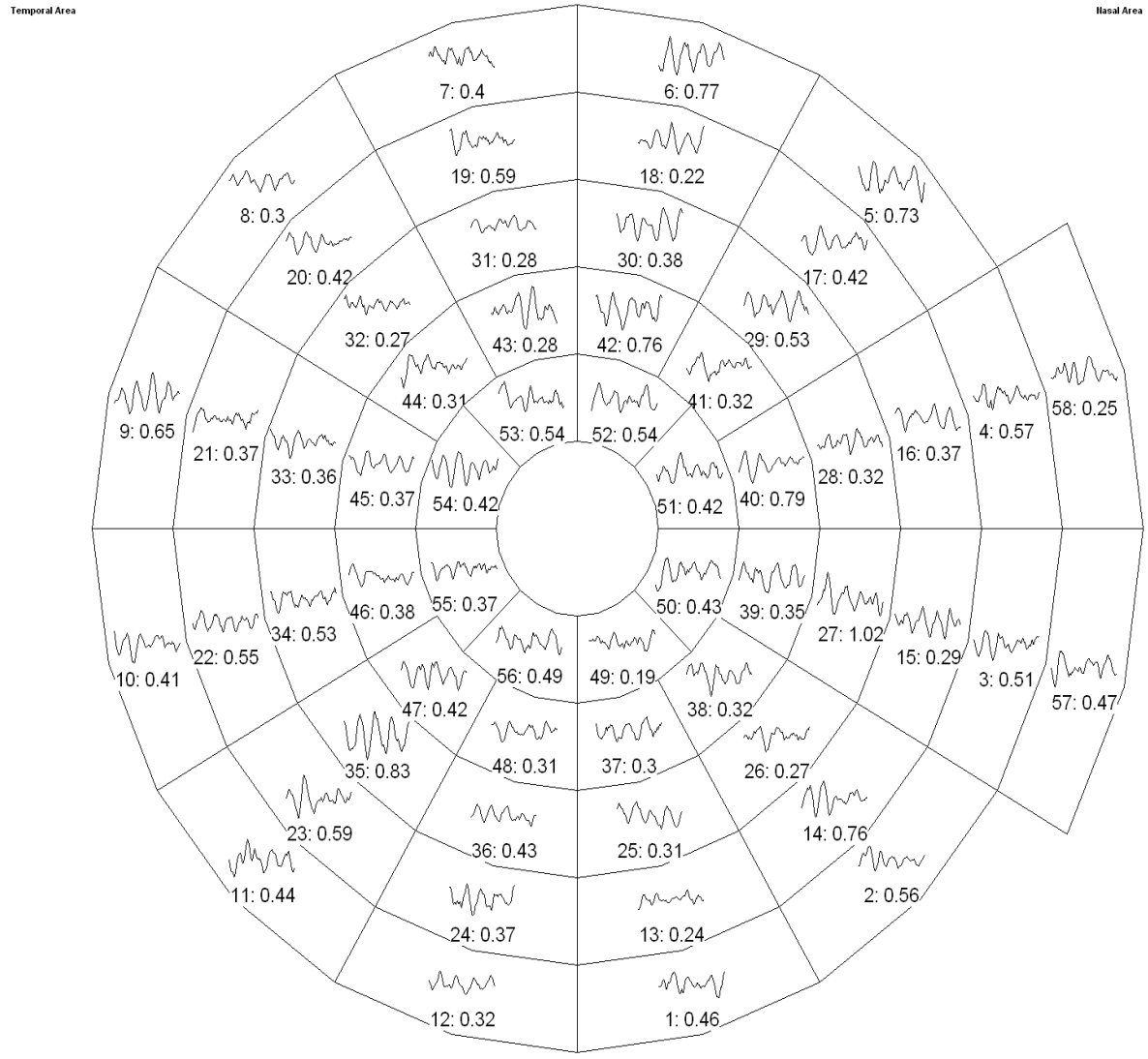



Figure (28) shows a good example of pure noise without any true signal recorded. This recording was obtained from a study subject with thick hair without proper contact with the scalp at any of the four contact points of the scalp electrode saddle. Attention should be paid for these recordings as looking carefully will reveal some eminent positive and negative components in the individual waveforms, which could be falsely interpreted as a true signal, specially that SNR values will be recorded and occasionally will give high values.

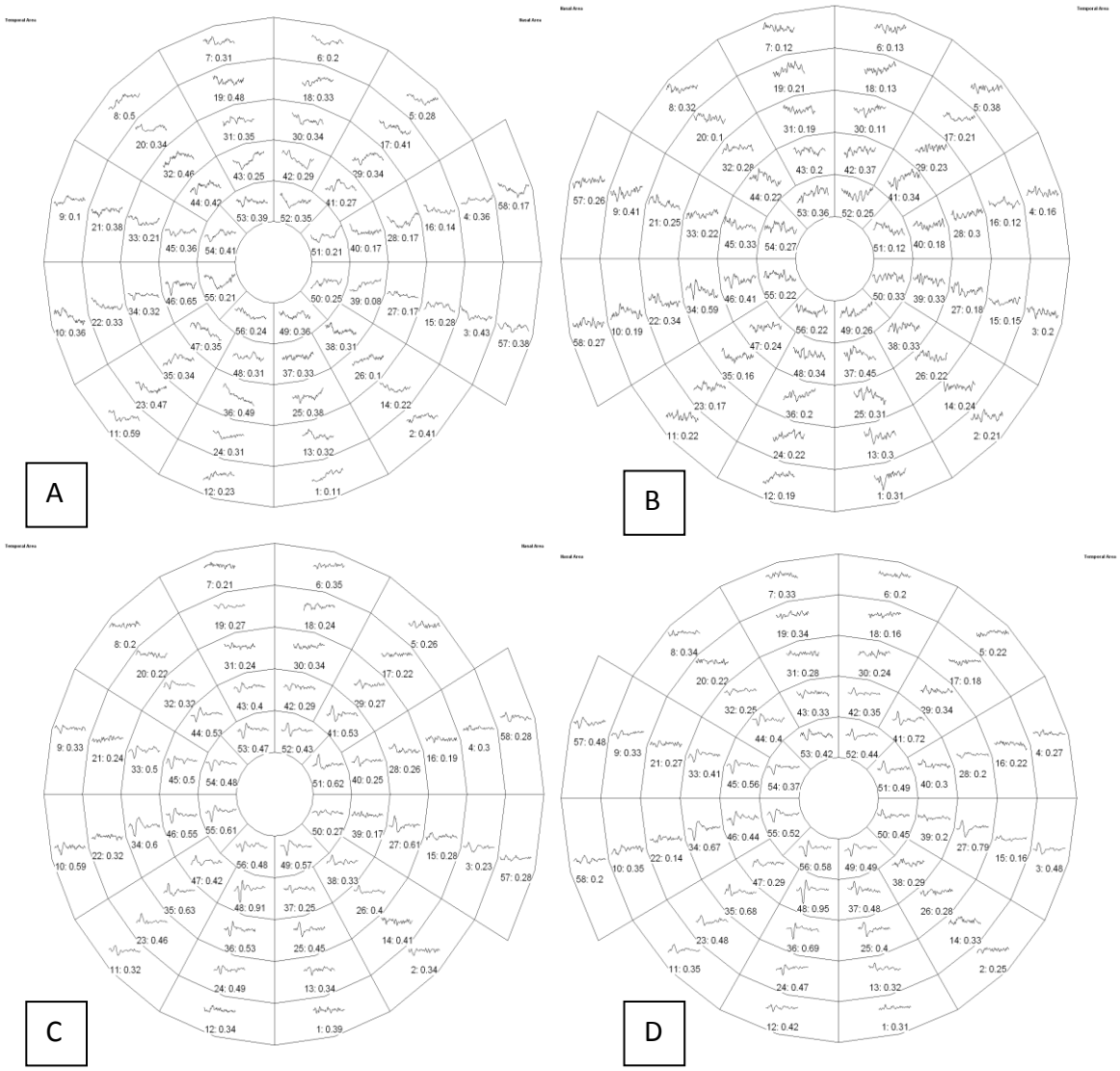
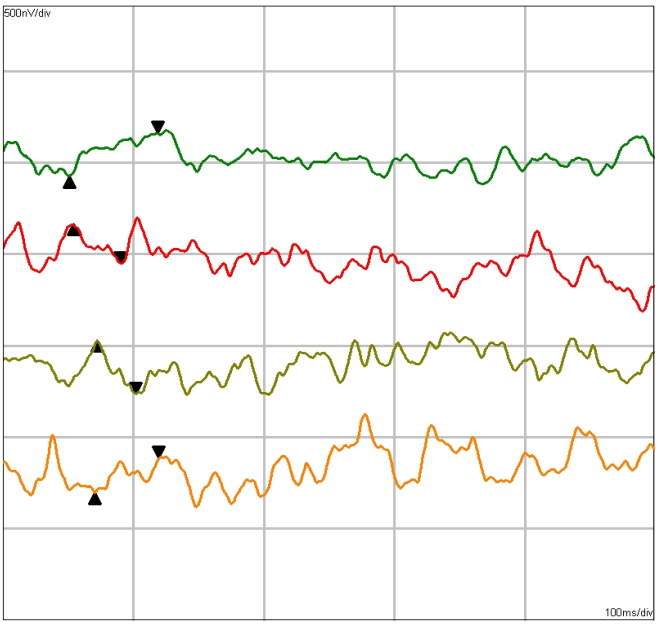



Figure (29) shows two examples of bad recordings; (A) is reduced responses all over the field for a normal subject, and (B) is very noisy responses in the whole field of a normal subject. The waveform recordings in (A) were abnormally flat and suspecious of bad connection rather than true reduction in responses. The waveforms in (B) were very irregular and full of noise masking a true signal in the entire field, which is a problem usually arises from amplifier connectivity to the electrodes. After rechecking the connections and recaliberating the amplifier output through impedence assessment, we were able to record better signals and good responses shown in both (C) and (D).




| Group | SNR [] | Amp.P1 [µV] | Amp.N1 [µV] | PeT.P1 [ms] | PeT.N1 [ms] |
|---|---|---|---|---|---|
| • 1 | **0.89** | 0.237 | 0.184 | 118.6 | 51.0 |
| • 2 | **1.05** | 0.215 | 0.068 | 90.2 | 53.9 |
| • 3 | **1.02** | 0.294 | 0.100 | 102.0 | 72.5 |
| • 4 | **1.04** | 0.191 | 0.134 | 119.6 | 70.6 |

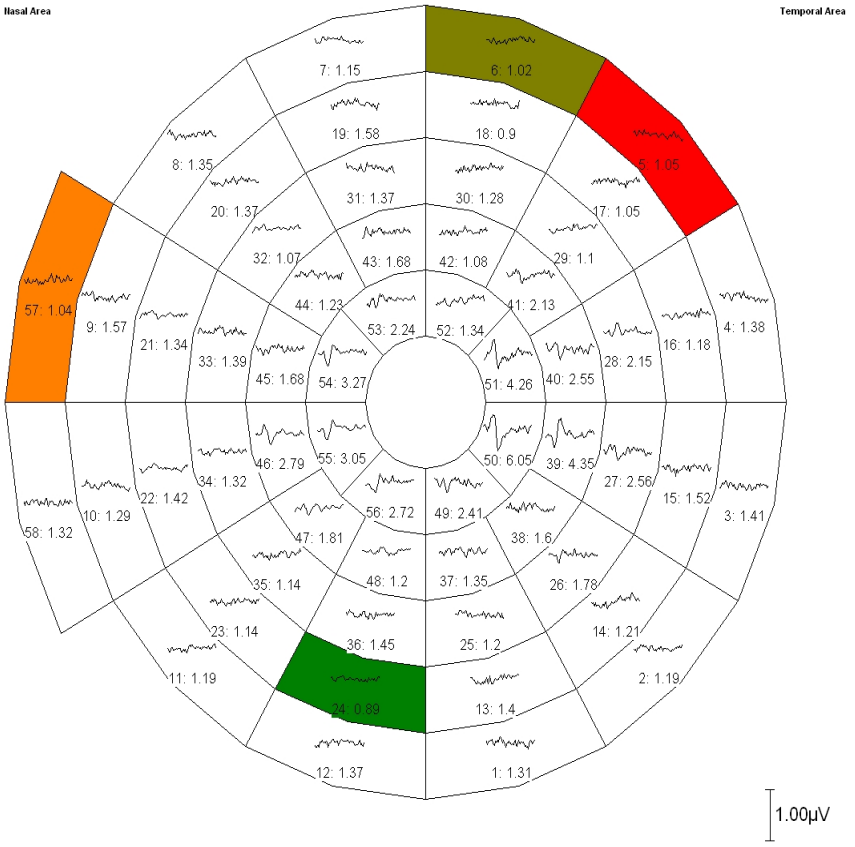



Figure (30) shows example of pure noise recordings in some segments, where the SNR value is <1 or almost equal to 1.0. Segment number (1) shows SNR value of 0.89; segment number (2) SNR = 1.05; segment number (3) SNR = 1.02 and segment number (4) SNR = 1.04. In the waveform graph on the left it is easily noted that there is no specific positive (P) and negative (N) components, just pure irregular noise is recorded. All segments with SNR values ≤ 1 were excluded from the average sector / hemi-ring calcuation.

### 3.6.3 Field variability of mfVEP recordings

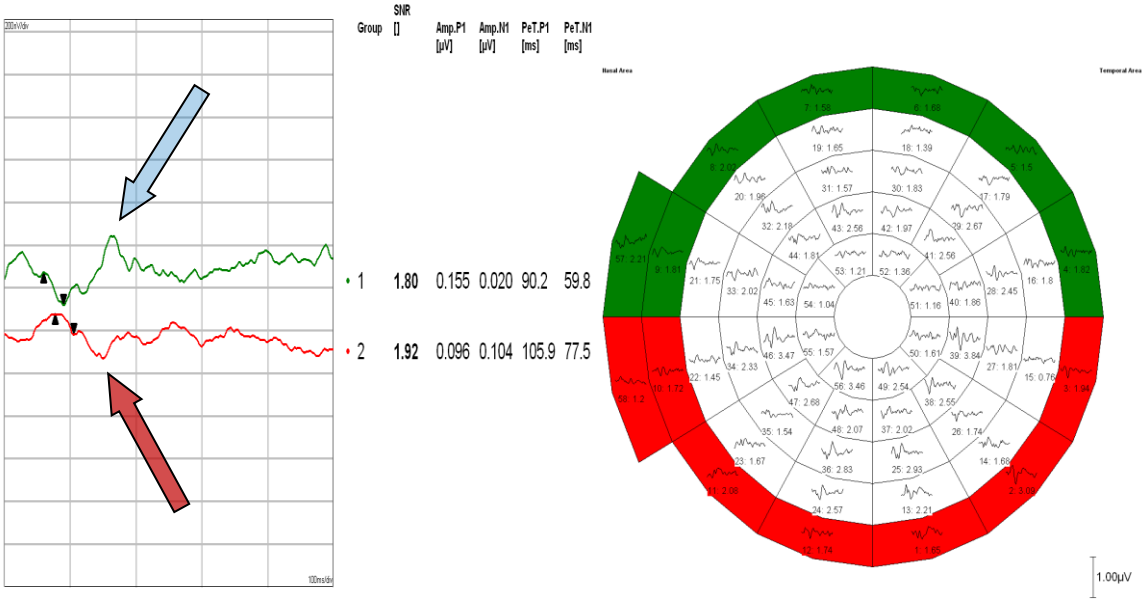



Figure (31) mfVEP hemi-ring analysis in a normal subject shows superior outer hemi-ring (SR1) compared to corresponding inferior hemi-ring (IR1). The two waveforms are reversed in polarity (arrows) which is a common normal finding in mfVEP recordings. There are few recordings doucmented in this study for normal subjects showed no polarity reversal across the horizontal meridian.

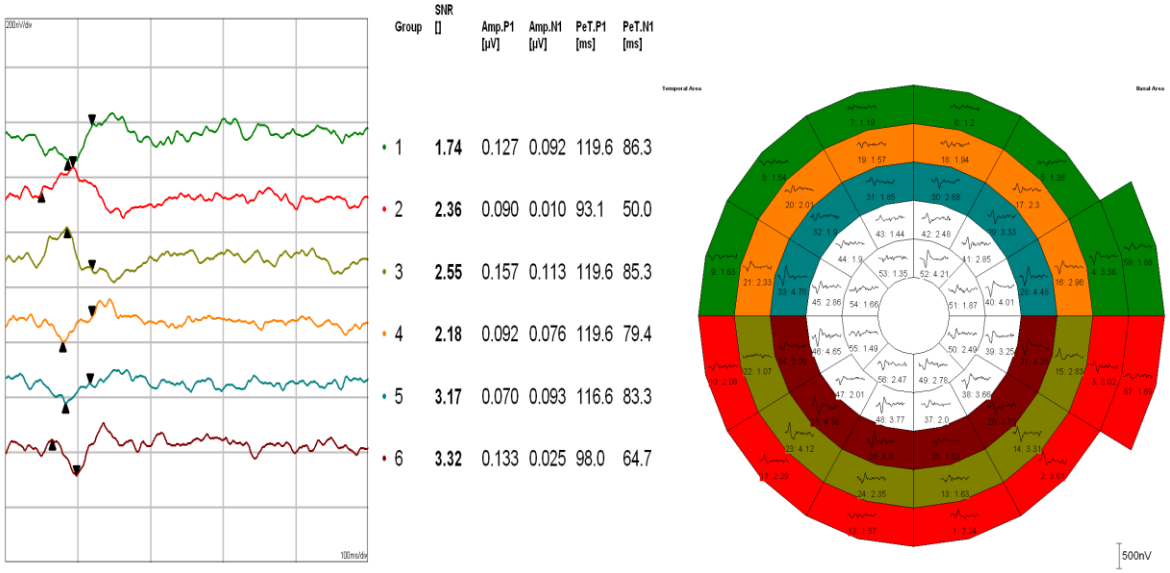



Figure (32) mfVEP hemi-ring analysis in a normal subject shows multiple eccentric hemirings in both superior and inferior hemifields. The SNR values in the outer segments were lower than the central. The waveform plots show that the more we move centrally the SNR increases significantly (from 1.74 in outer hemi-ring to 3.32 to the inner most hemi-ring). This is a common normal finding in the mfVEP test printout.

## 3.6.4 Role of HSA protocol in identifying normal recordings

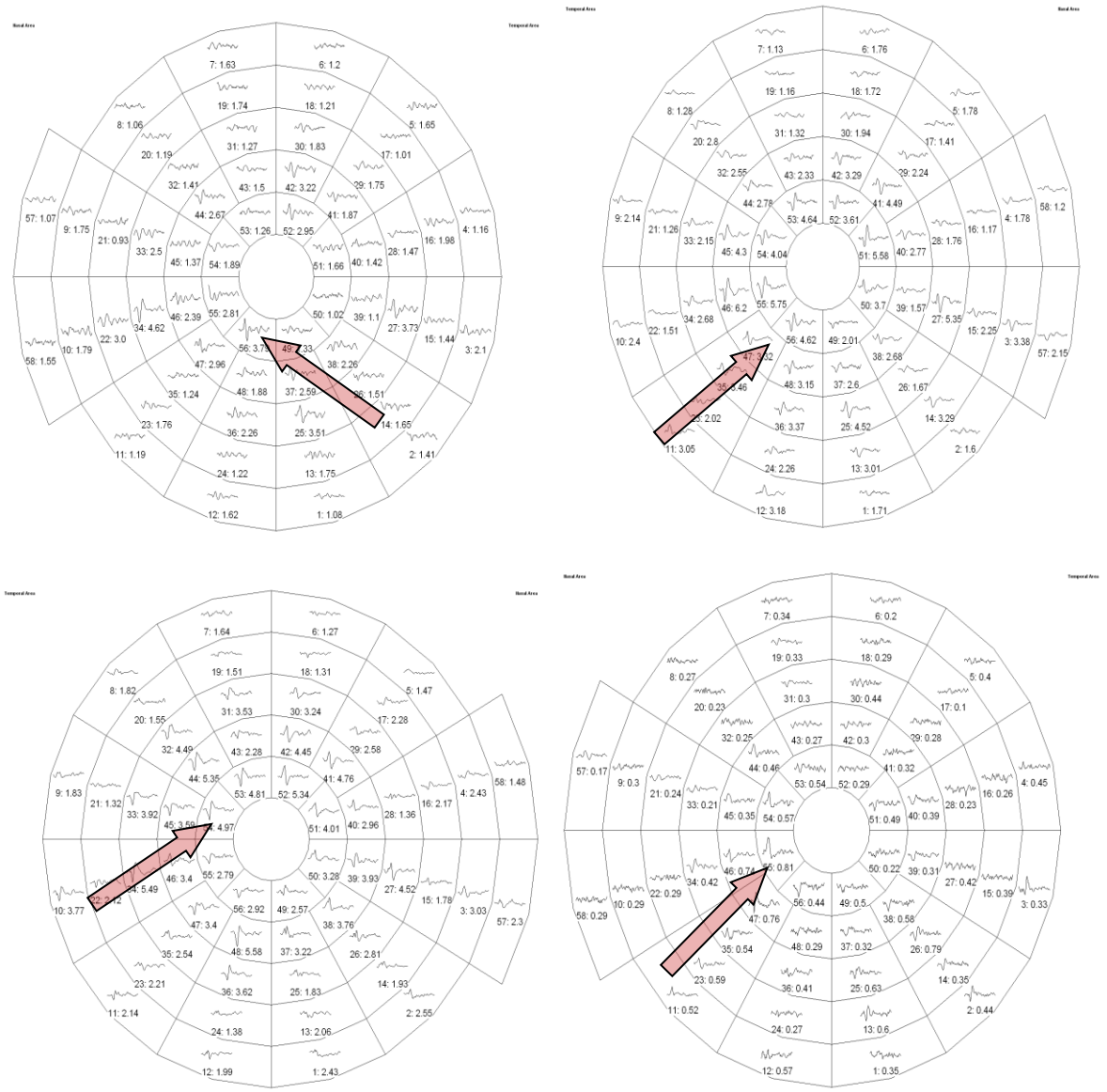
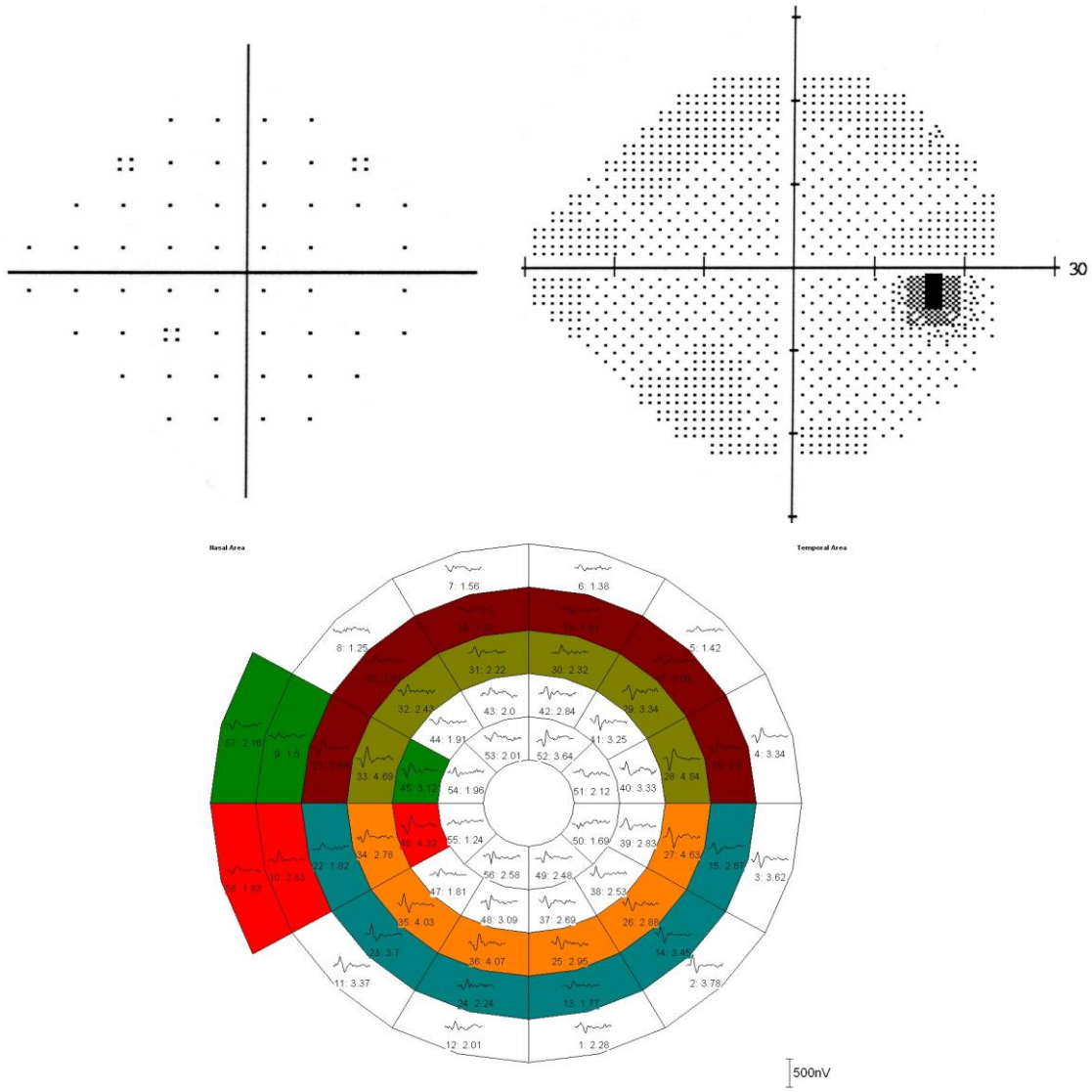
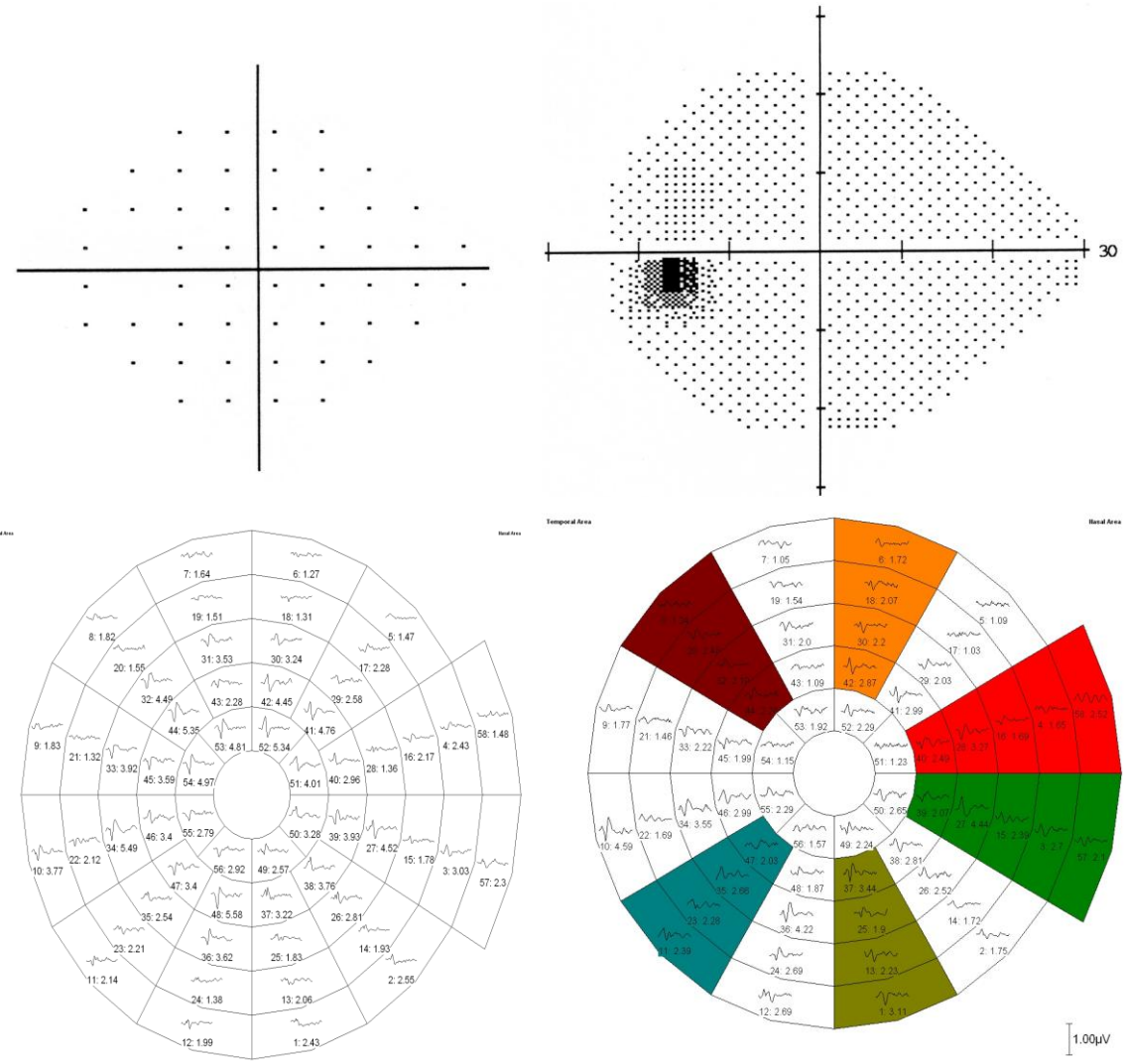


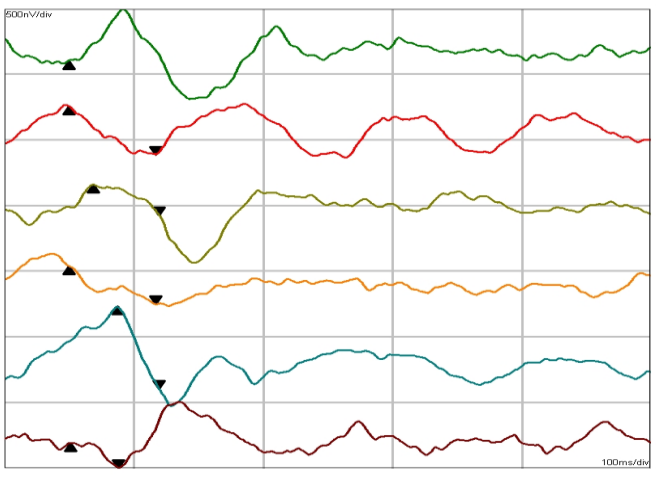
Figure (33) shows 4 examples of mfVEP recordings from normal subjects. There are variable forms of normal responses related to the shape, amplitude and latency of the signal that can reflect the normal variation among population. It is clear that peripheral segments usually smaller and more irregular when compared to central segments. Also the location of foveal response is variable among individuals according to the relative relation between the recording electrodes and the convoluted visual cortex. These locations were identified as the foveal response as it shows the highest central response in all 4 channel recordings. (The arrows showing the variable locations of the fovea)




| Group | SNR [] | Amp.P1 [µV] | Amp.N1 [µV] | PeT.P1 [ms] | PeT.N1 [ms] |
|---|---|---|---|---|---|
| 1 | **3.03** | 0.189 | 0.007 | 93.1 | 50.0 |
| 2 | **2.72** | 0.152 | 0.014 | 100.0 | 67.6 |
| 3 | **3.31** | 0.137 | 0.092 | 119.6 | 82.4 |
| 4 | **3.56** | 0.166 | 0.124 | 119.6 | 77.5 |
| 5 | **2.61** | 0.145 | 0.095 | 118.6 | 77.5 |
| 6 | **2.17** | 0.065 | 0.029 | 84.3 | 53.9 |

Figure (34) mfVEP recordings with sector / hemi-ring analysis compared to SITA standard 24-2 printout (greytone and pattern deviation plots) of a normal subject. The visual field is within normal limits with no signifcant reproducible defects. The HSA analysis shows symmetrical SNR values between the two hemifields in sectors and hemi-rings (bottom) numbered from 1-6 in the waveform box. The two tests show identical results and good agreement. The average SNR values were high average normal, ranged between (2.17-3.56) in the entire visual field.




| Group | SNR [] | Amp.P1 [µV] | Amp.N1 [µV] | PeT.P1 [ms] | PeT.N1 [ms] |
|---|---|---|---|---|---|
| 1 | **2.74** | 0.395 | 0.109 | 92.2 | 50.0 |
| 2 | **2.33** | 0.370 | 0.270 | 116.7 | 50.0 |
| 3 | **2.67** | 0.237 | 0.202 | 119.6 | 68.6 |
| 4 | **2.21** | 0.295 | 0.079 | 116.7 | 50.0 |
| 5 | **2.34** | 0.633 | 0.542 | 119.6 | 87.3 |
| 6 | **2.04** | 0.190 | 0.052 | 88.2 | 51.0 |

Figure (35) shows mfVEP recordings with sector / hemi-ring analysis compared to SITA standard 24-2 printout (greytone and pattern deviation plots) of a normal subject. The visual field is within normal limits with no signifcant reproducible defects. The HSA analysis shows symmetrical SNR values between the two hemifields in random sectors (middle right) numbered from 1-6 in the waveform box (below). The two tests shows identical results and good agreement. The SNR values were high average normal (ranged between 2.04-2.74) in the entire visual field.

## 3.6.5 Role of HSA protocol in identifying glaucomatous visual field defects

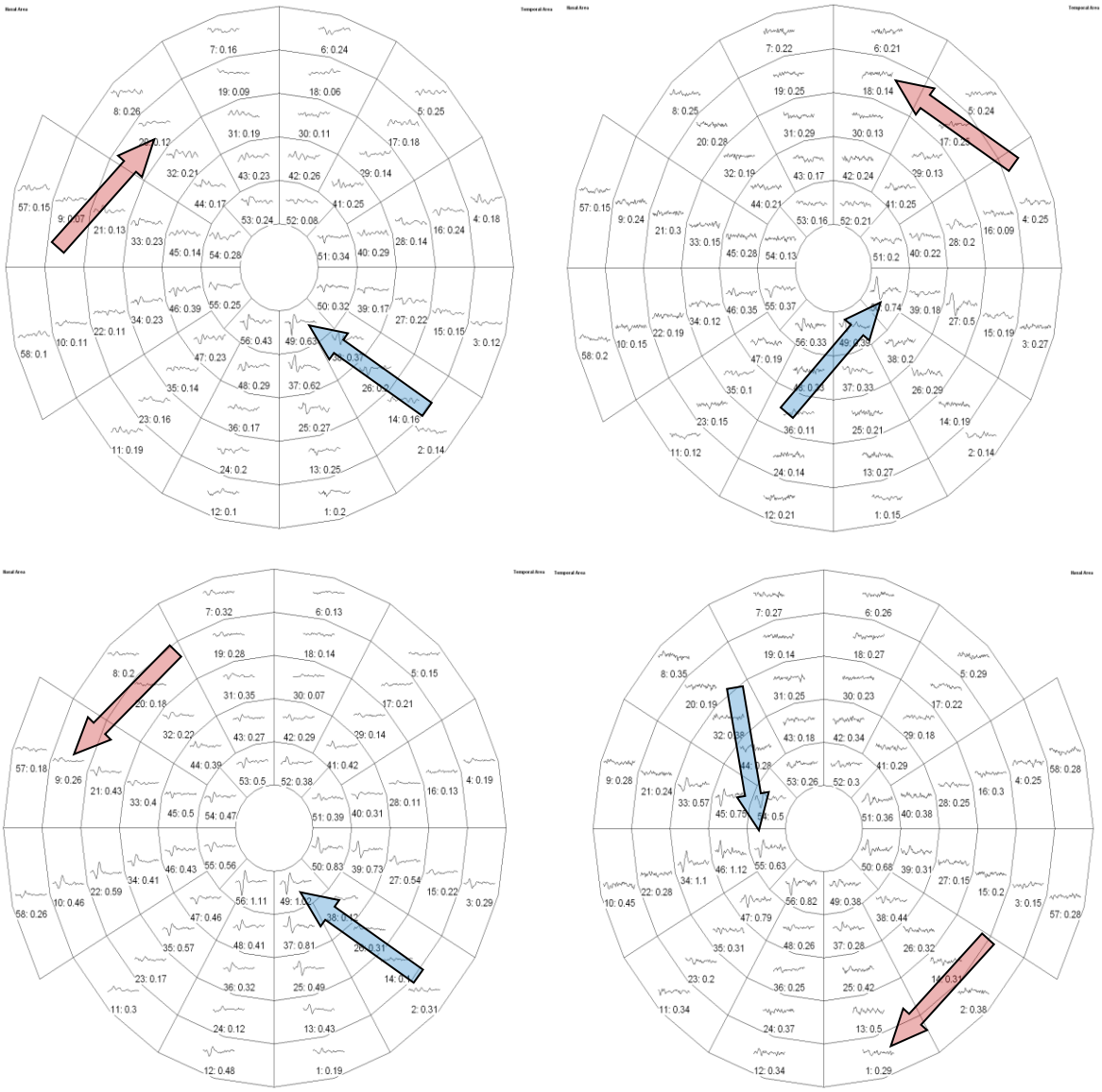


Figure (36) shows 4 examples of glaucoamtous visual field depressions in the mfVEP recordings. The field is constricted, in a classic periphero-central pattern. Red arrows show the peripheral depressed signals, while blue arrows show central preserved good responses. The waveform representation in addition to the SNR values of each segment clearly confirm the low responses from the affected field locations.

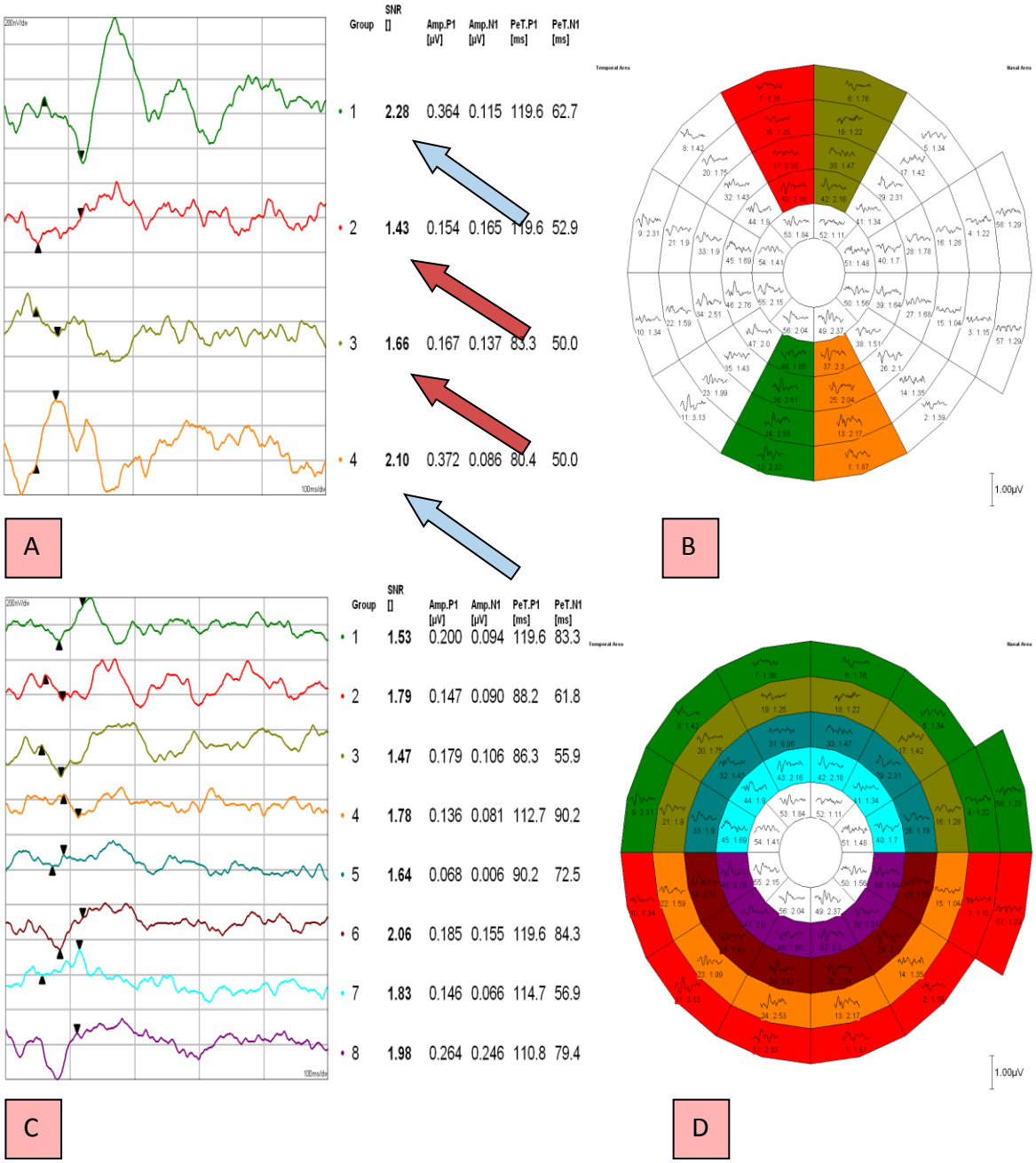



Figure (37) highlight the need to perform both sector and hemi-ring analysis together to identify focal defects. The superior HSA printout (A,B) shows superior focal sector depression (red and brown sectors) with low SNR values (red arrows), compared to their corresponding sectors in the inferior hemifield (green and orange) which show signifcantly higher SNR values (blue arrows). When the hemi-ring analysis was carried out on the same eye, these focal depressions were missed due to the variations in the averaged segments between the sectors and hemi-rings. The lower printout (C,D) shows almost similar SNR values between superior and inferior hemi-rings with no evidence of focal asymmetry across the horizontal midline.

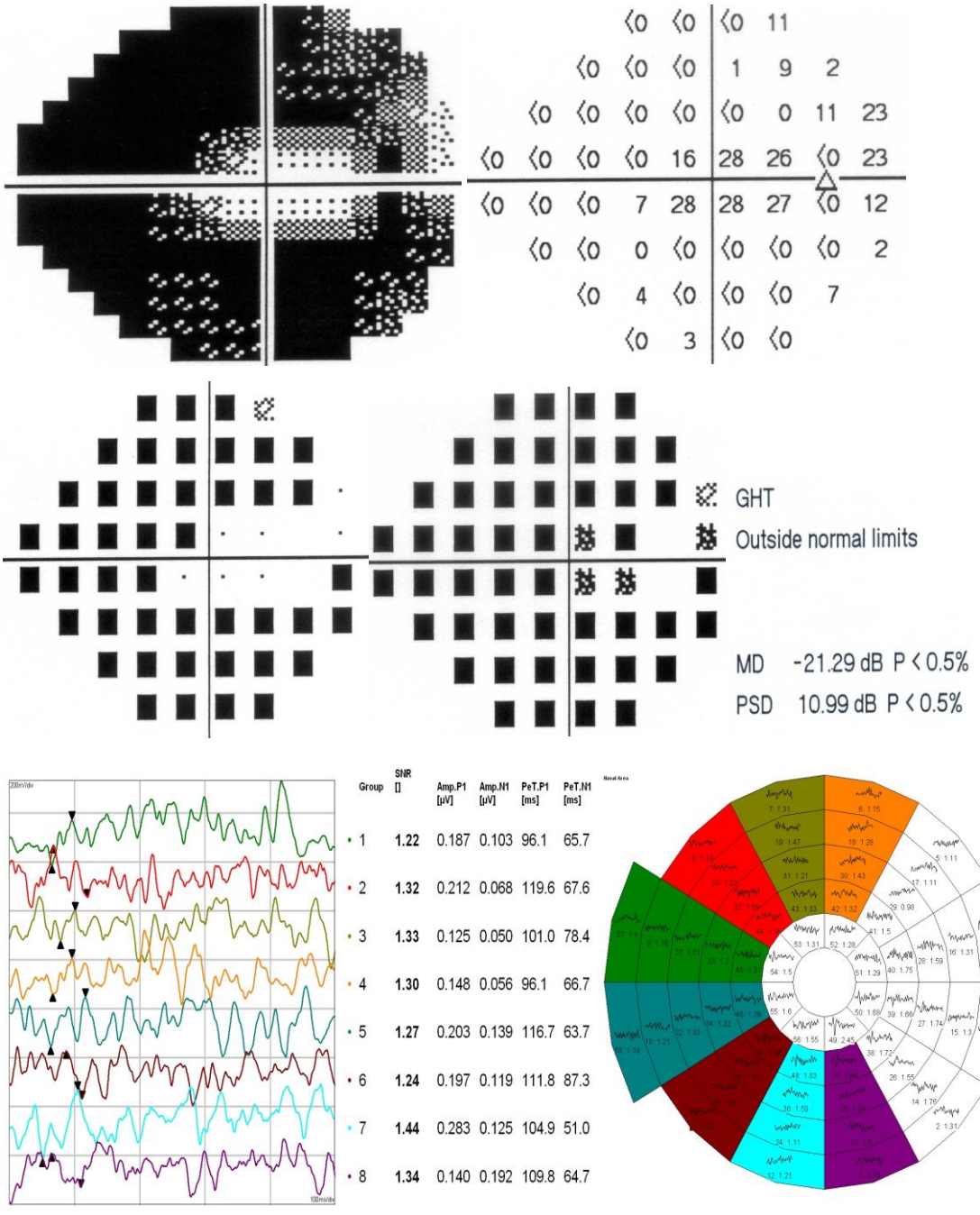



Figure (38) shows an example of agreement between the SITA standard results and mfVEP sector analysis protocol in a patient with advanced glaucomatous visual field defect. The visual field printout shows constricted field with only central $5^{o}$-$7^{o}$ island preserved. The HSA shows that corresponding field defects are identified on the printout as very poor (noisy) waveform configurations and responses, with G5 SNR values (ranged between 1.22 and 1.44) shown in the waveforms numbered from 1 to 8. This patient did not show significant intersector SNR difference because all corresponding sectors in both hemifields show symmetrically low SNR values. In these patients only the SNR values and its grade should give a good indication of glaucomatous field defects without intersector comparison.

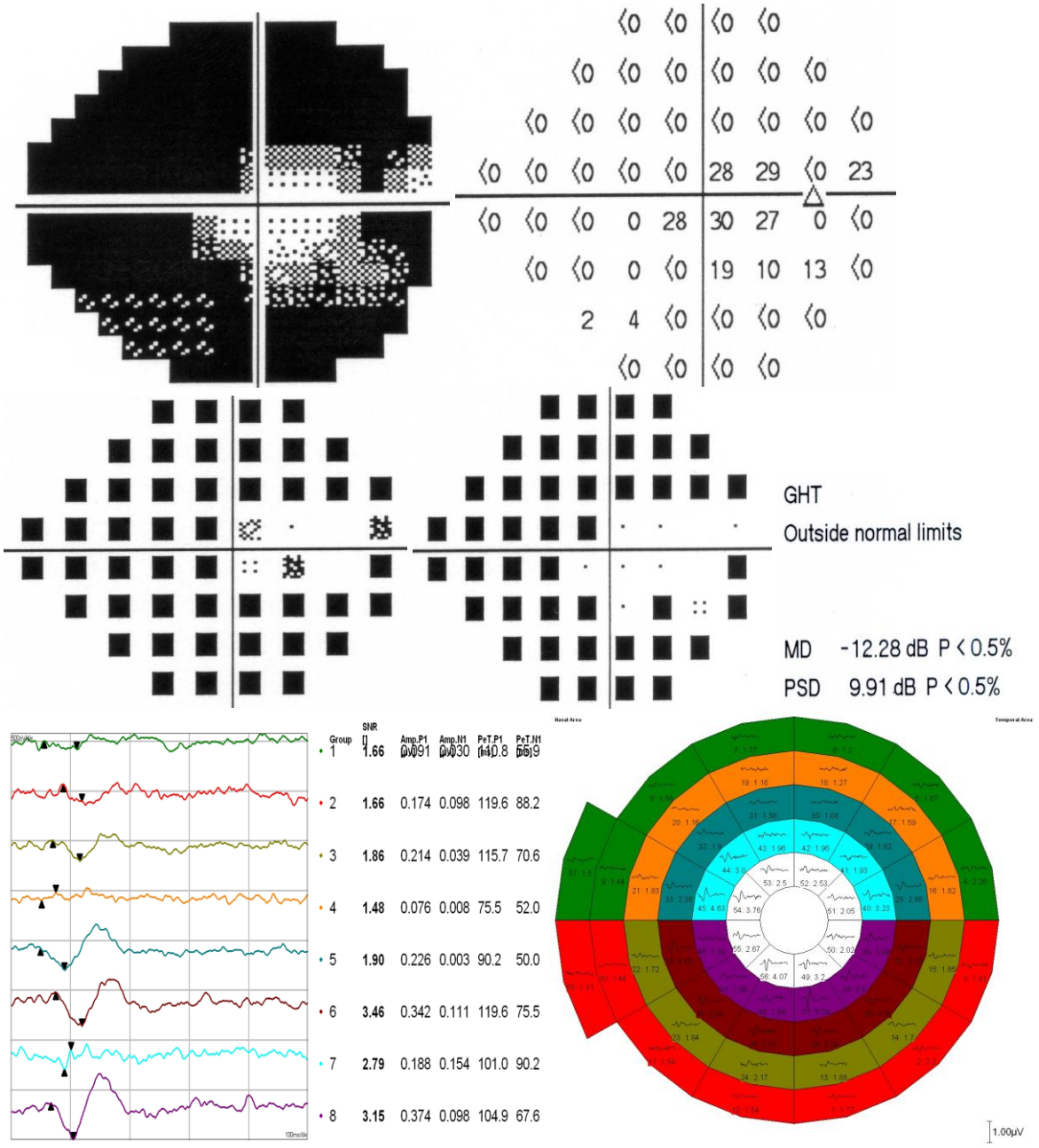



Figure (39) shows another example of good agreement between the SITA standard results and mfVEP sector analysis protocol in a patient with advanced glaucoamtous visual field defect. The visual field printouts show a constricted field with partial central involvment and preserved central island. The HSA shows that corresponding field defects are identified on the printout as very poor waveform configurations and responses, with G4-G5 SNR values (ranged between 1.48 and 1.90) shown in the outer hemi-fings numbered from 1 to 5. The central hemi-rings (numbered 6-8) are preserved and show G1-G2 SNR values (2.79-3.46). This patient did not show significant hemi-ring SNR difference because all corresponding hemi-rings in both hemifields are symmetrically low SNR values. In these patients only the SNR values and its grade should give a good indication of glaucomatous field defects without intersector comparison.

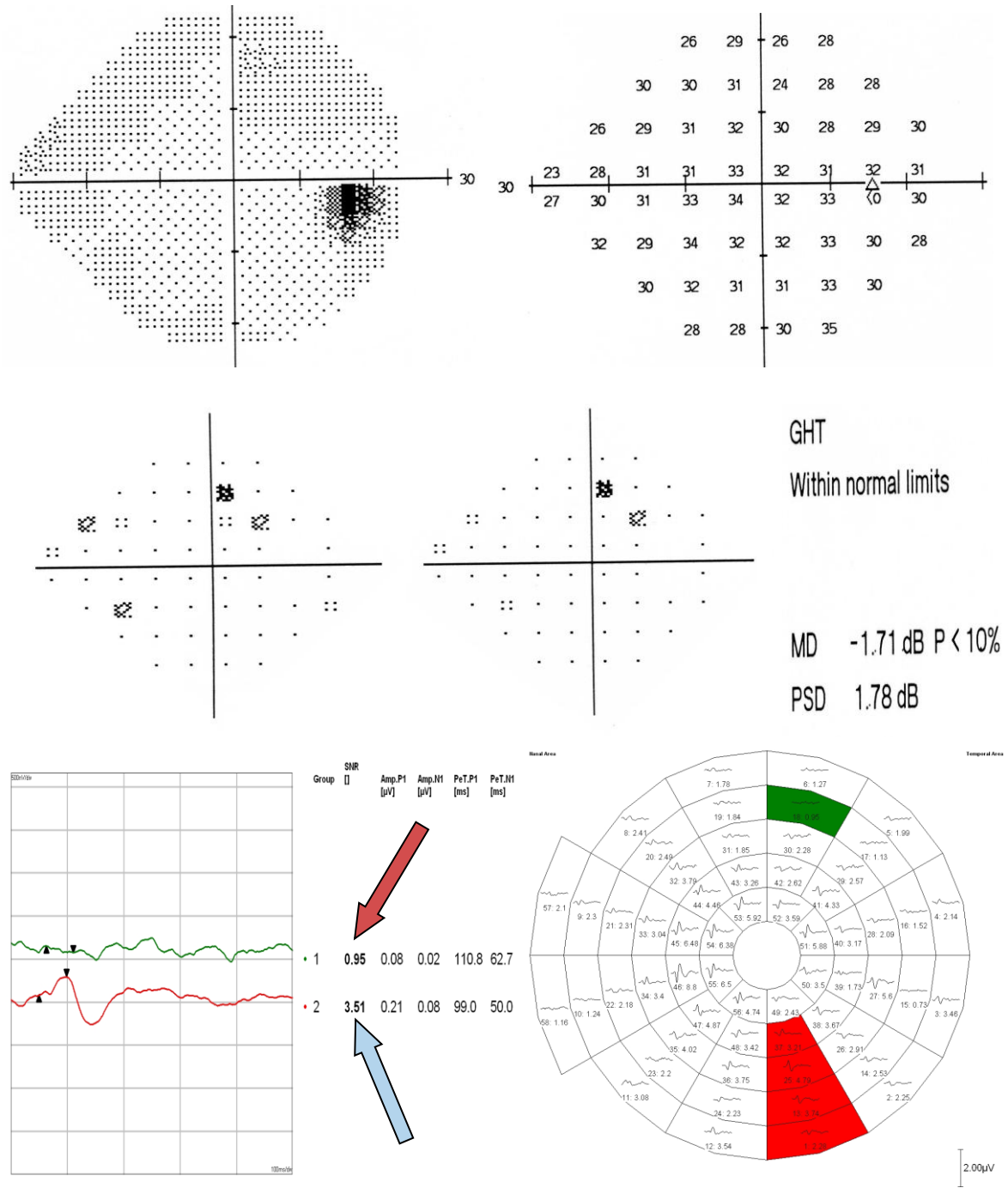



Figure (40) shows focal scotoma in a glaucoma suspect patient detected by using the mfVEP HSA Intersector analysis in the superior hemifield when compared to its corresponding location in the opposite hemifield. The waveform configuration clearly shows the big SNR difference between superior scotoma (0.95) and the inferior sector average (3.51). The SITA 24-2 test shows the scotoma location more diffuse and not localized as the mfVEP. However, despite the multiple deep defects in the pattern deviation plot, the GHT showed “within normal limits”. The combination of the two tests can confirm the defect and prompt the clinician that most likely it is a true glaucomatous defect.

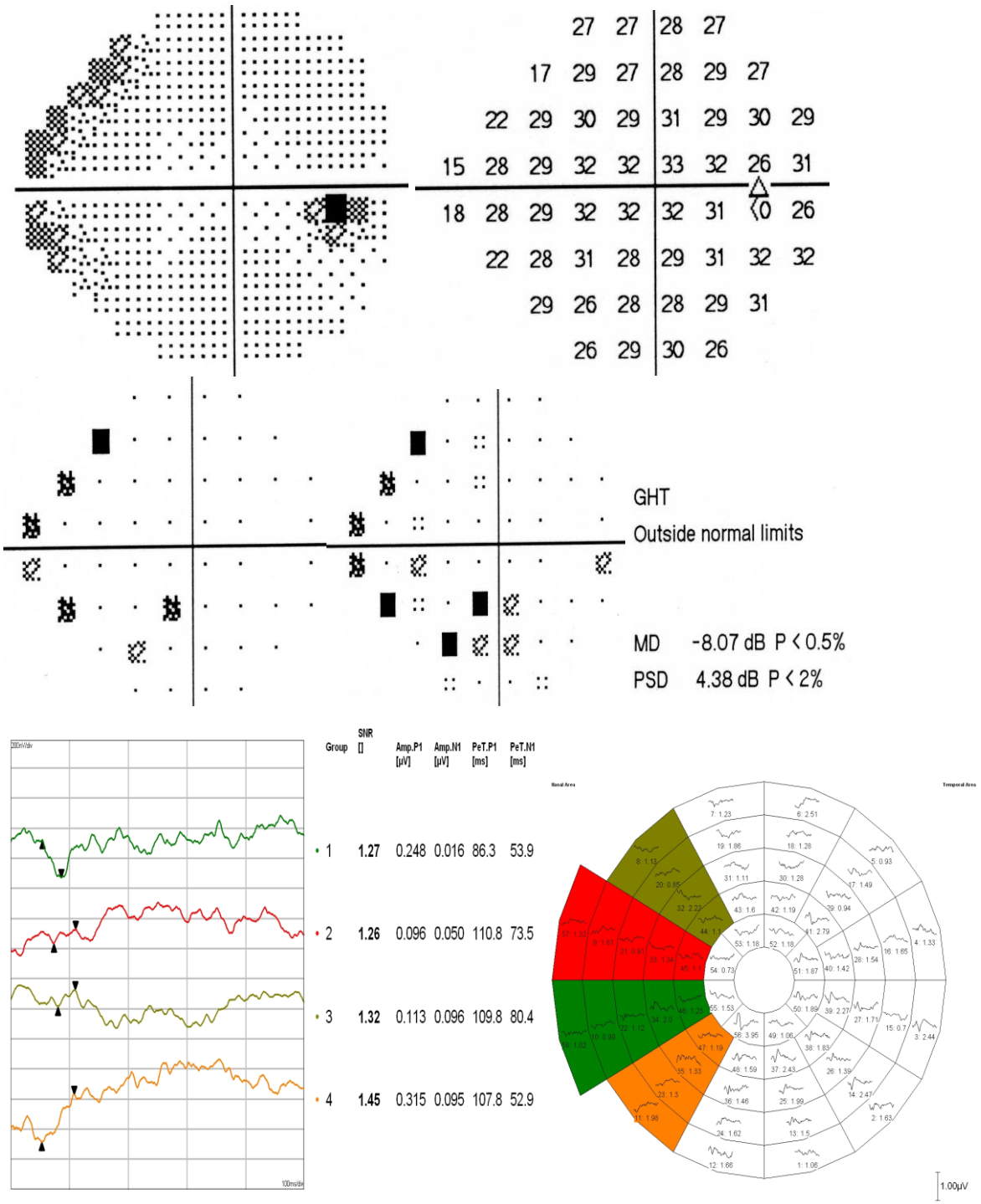



Figure (41) shows good agreement between SITA standard 24-2 and mfVEP intersector analysis in a glacuoma patient. Both tests confirm nasal stepping in both. The HSA results show significant signal reduction (G5 SNR values ranged between 1.26 and 1.45) in the nasal sectors. The rest of the visual field in both tests shows fairly preserved sensitivity, matched by good signal configuration in the mfVEP plot.

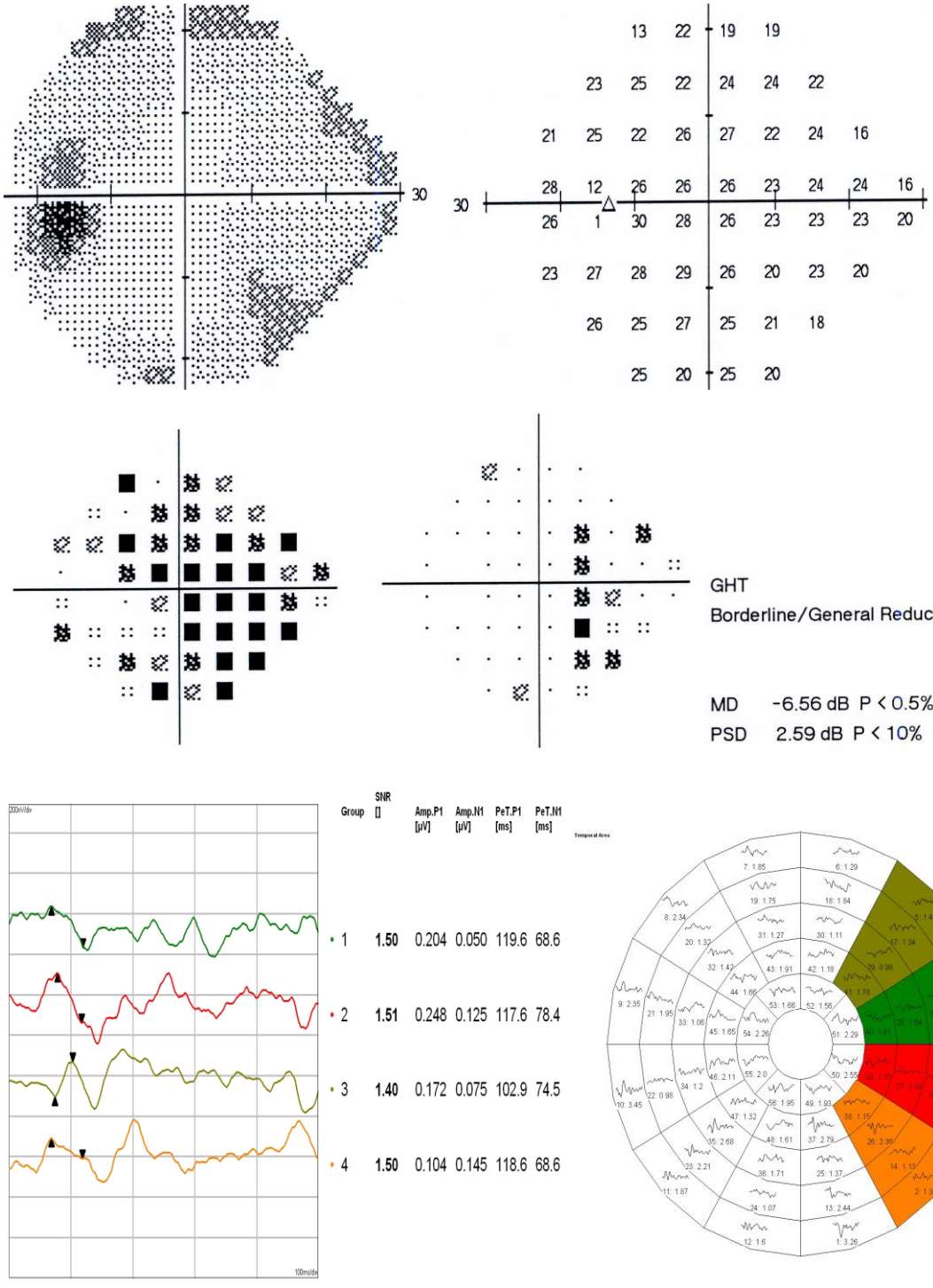



Figure (42) shows SITA standard 24-2 test and mfVEP intersector analysis for a glaucoma patient. The SITA test shows generalized reduction in sensitivity shown in the total deviation plot and in the GHT due to early cataract, and localized nasal stepping defects in the pattern deviation plot showed as borderline in the GHT. mfVEP intersector analysis confirmed the localized defect in its location and severity (SNR avergae G5 from all nasal sectors). The intersector analysis can confirm field defects when it is masked by other causes of field dpressions such as generalized reduction in sensitivity due to early cataract. The addition of mfVEP analysis can be beneficial in these patients.

## 3.6.6 Role of HSA protocol in identifying early visual field changes

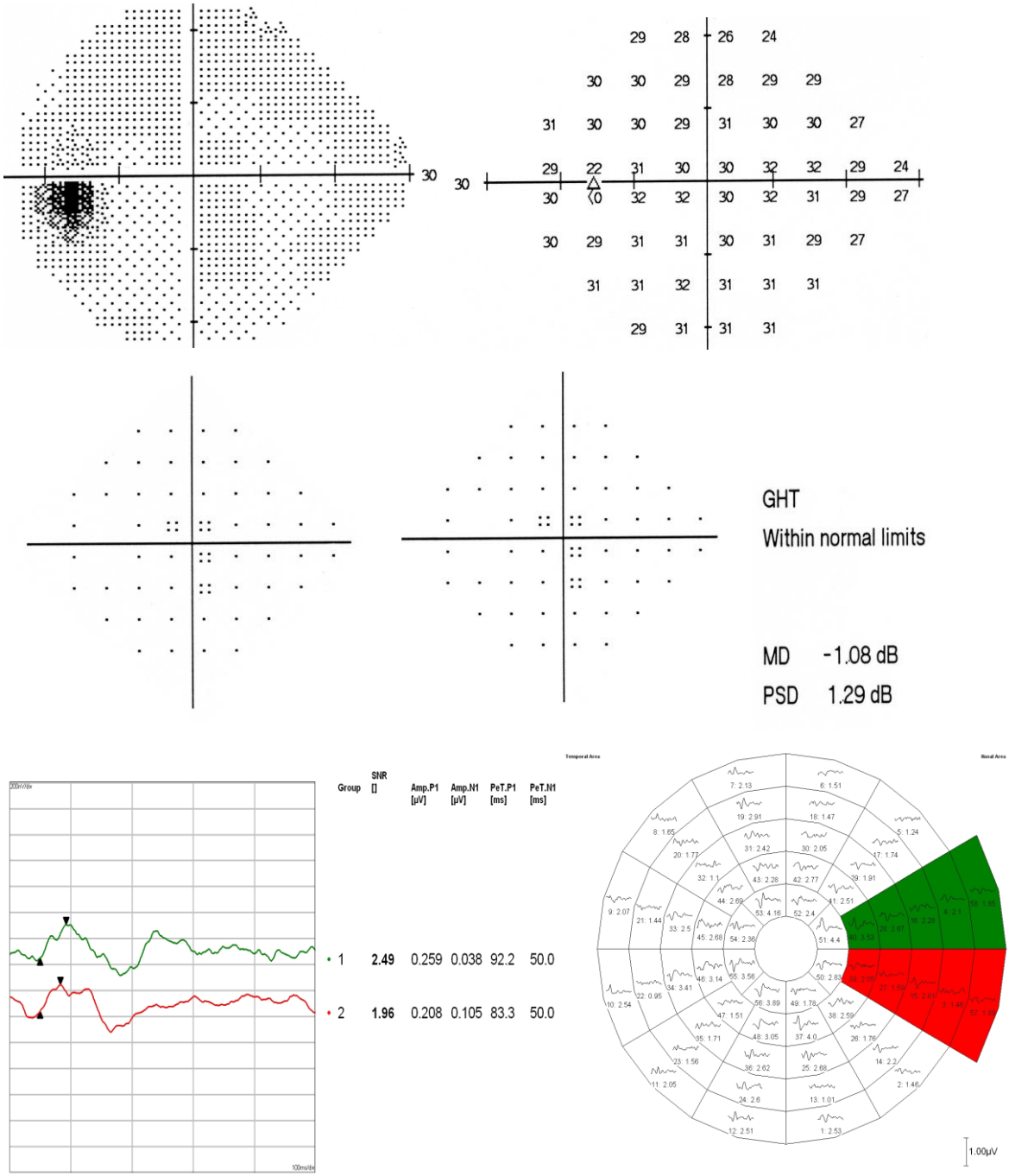



Figure (43) shows SITA standard 24-2 test and mfVEP intersector HSA comparison in a glaucoma suspect patient. No signifcant visual field defects recorded in the SITA test; neither in the total deviation nor the pattern deviation plots. The GHT defined the visual field as “within normal limits”. The intersector analysis showed signifcant difference between the nasal sectors (SS1 = 2.49 and IS1 = 1.96) pointing to early inferior reduced response. This difference can reflect an early functional change in the visual field that could not be detected by the SITA standard test.

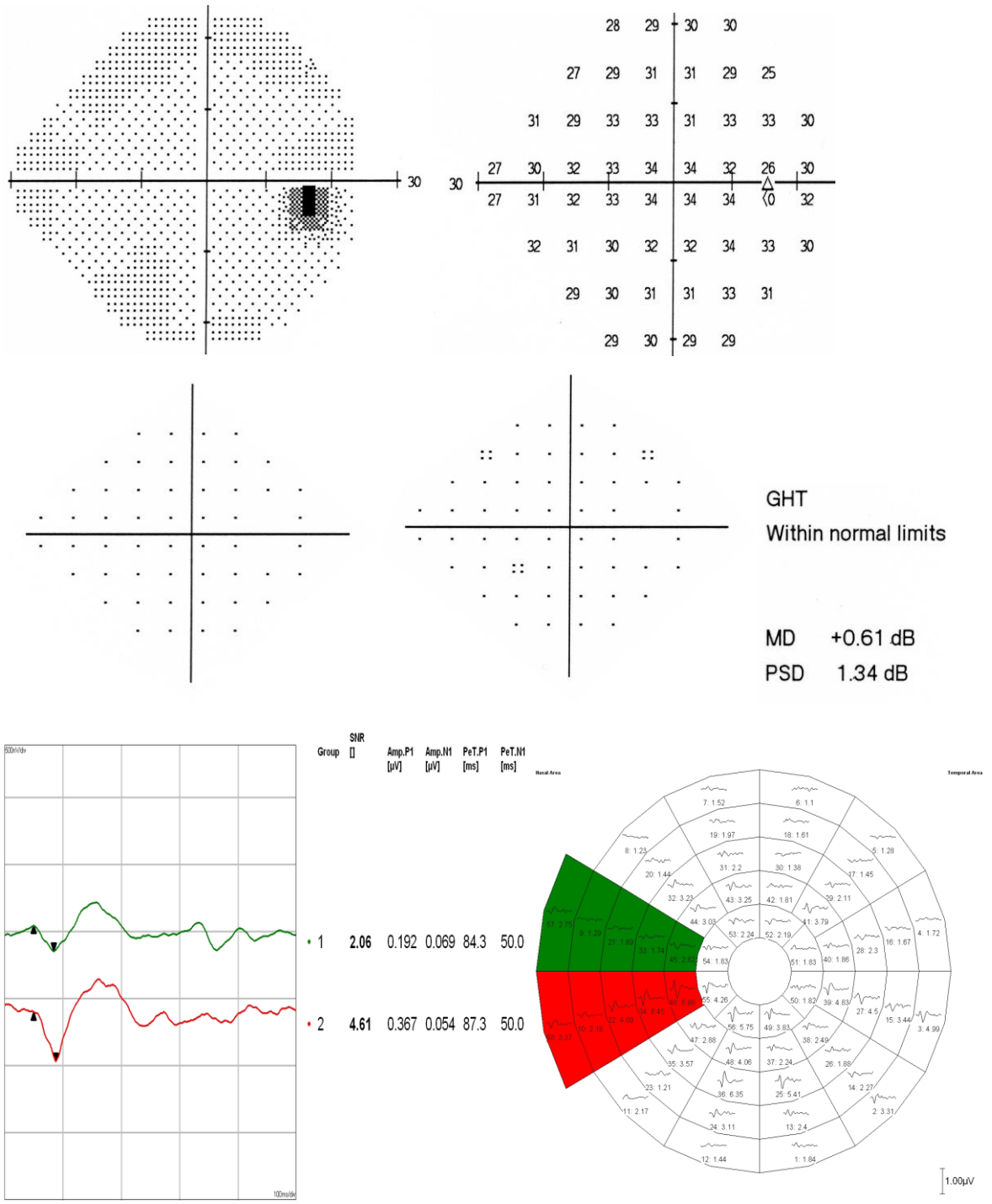



Figure (44) shows another example of possible early detection of visual field defect. SITA standard 24-2 test and mfVEP intersector HSA comparison in a gluacoma suspect patient. No signifcant visual field defects recorded in the SITA test; neither in the total deviation nor the pattern deviation plots. The GHT was “within normal limits”. The intersector analysis showed signifcant difference between the nasal sectors (SS1 = 2.06 and IS1 = 4.61) pointing to possible early superior reduced response. This difference can reflect an early functional change in the visual field that could not be detected by the SITA standard test

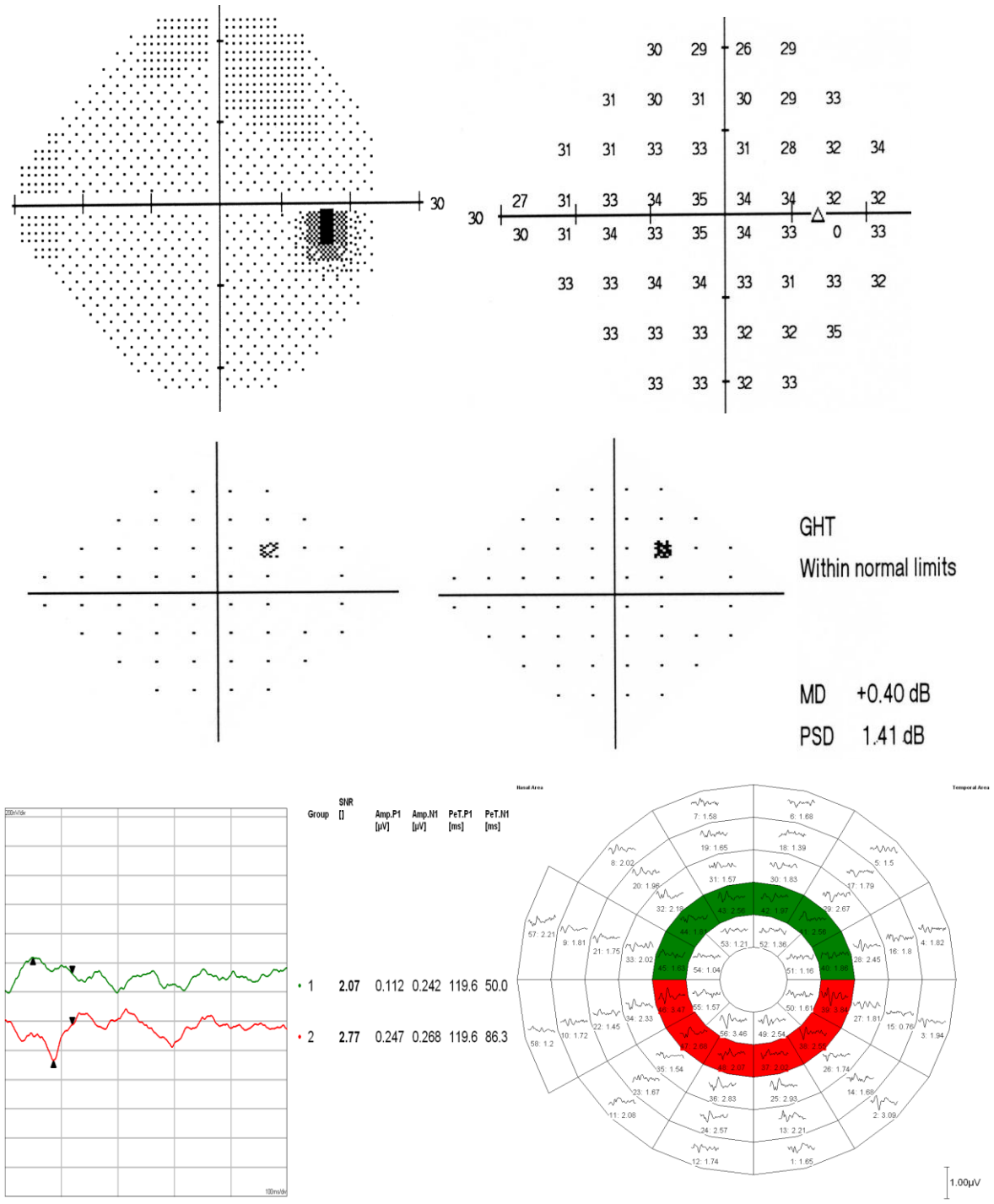



Figure (45) shows an example of possible early detection of visual field defect. SITA standard 24-2 test and mfVEP hemi-ring comparison analysis in a gluacoma suspect patient. No signifcant visual field defects were recorded in the SITA test. Some focal depression is present in the pattern deviation plot with a single location in the total deviation plot. The GHT was “within normal limits”. The inner hemi-ring (SR4-IR4) analysis showed signifcant difference between the superior and inferior hemi-rings (SR4 = 2.07 and IR4 = 2.77) pointing to possible superior reduced response. This difference may reflect an early functional change in the visual field that could not be detected by the SITA standard test

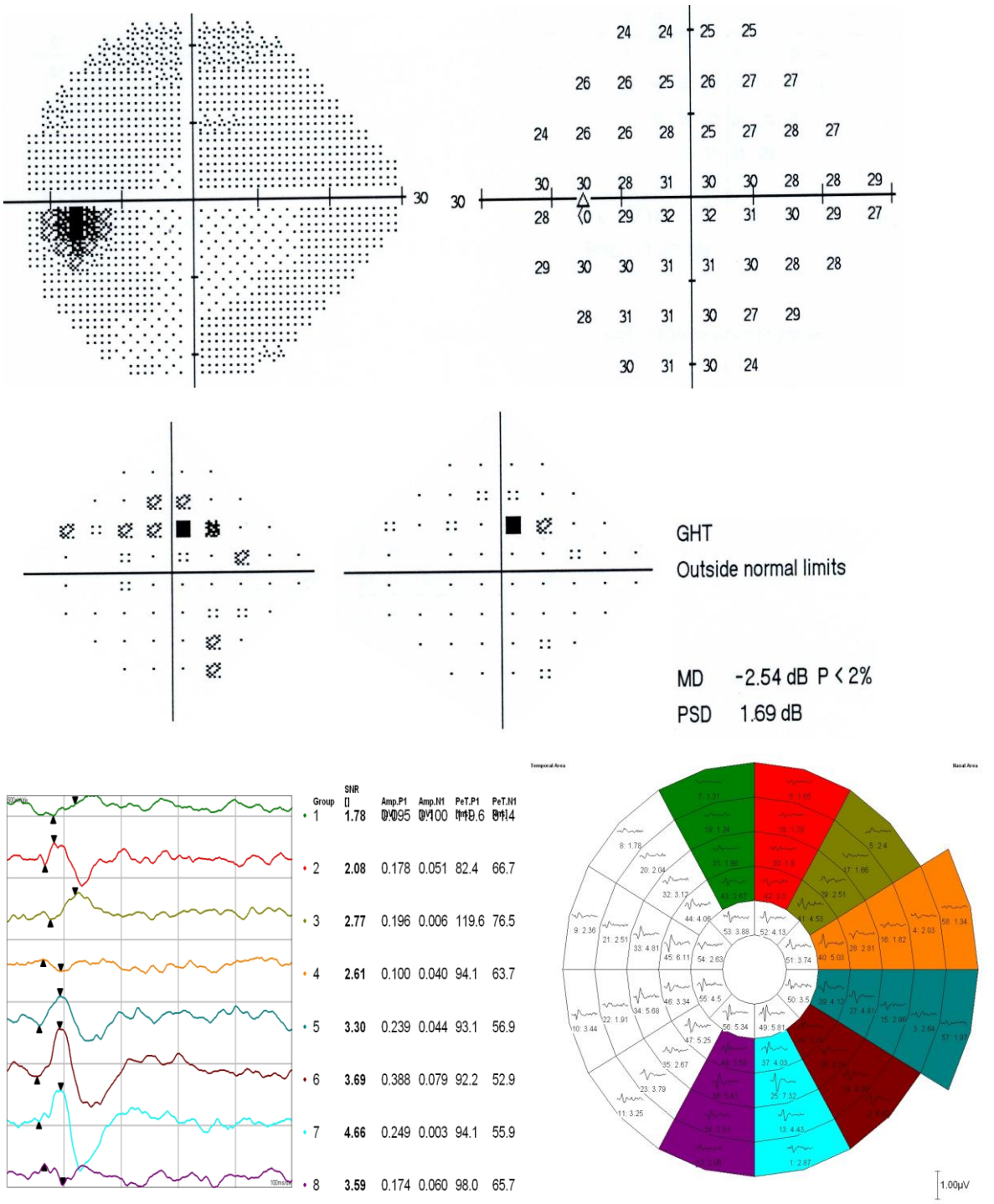



Figure (46) shows a SITA standard 24-2 test and mfVEP test with HSA printout for a glaucoma patient with a moderate glaucomatous visual field defect. The SITA test identified a diffuse cluster of signifcant defect shown in both total and pattern deviation plots, and confirmed by the GHT. The intersector hemifield analysis confirmed the defect, where it shows the superior 4 sectors (numbered 1-4) with lower SNR values compared to their inferior corresponding ones (numbered 5-8). This agreement confirm the role of mfVEP intersector analysis in the detection of visual field defects.

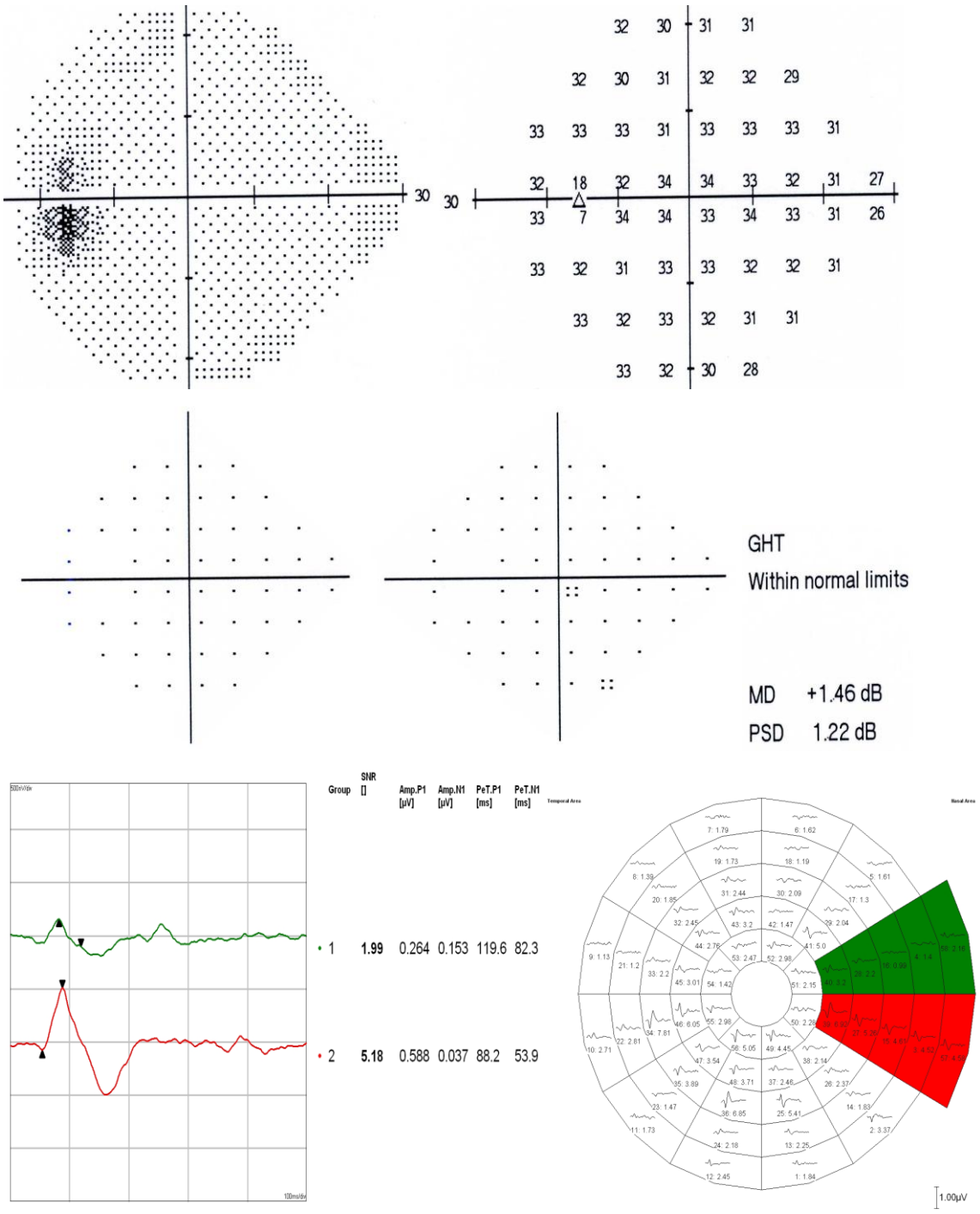



Figure (47) shows a SITA standard 24-2 test and mfVEP test with HSA printout for a glaucoma suspect patient without any signifcant field defects. GHT was “within normal limits”. The mfVEP intersector hemifield analysis shows signifcant superior nasal reduction in SNR value compared to its corresponding fellow sector in the inferior hemifield. SS1 shows SNR value 1.99 (G4) and IS1 SNR value is 5.18 (G1), indicating a possible superior nasal visual field defect. Perhaps a repeated and reproducible mfVEP defect, evidenced by the intersector analysis, can give an indication that this defect is true and was detected at an earlier stage before SAP test can detect it.

## 3.6.7 Benefit of combining SAP and HSA in suspicious visual field defects

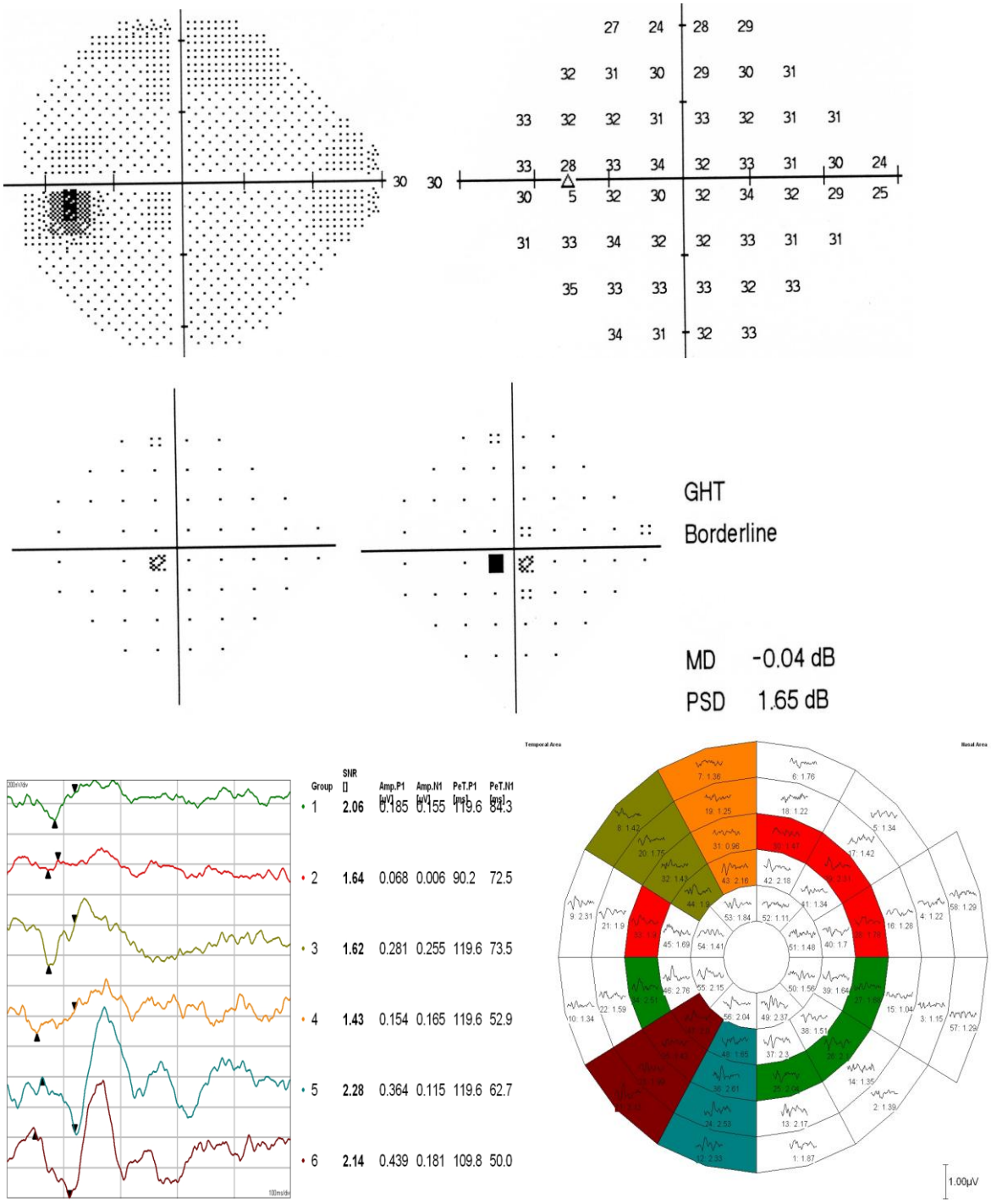



Figure (48) shows a suspicious SITA standard 24-2 test with central cluster of high significance shown in the pattern deviation plot and GHT "borderline". The mfVEP intersector and hemi-ring analysis shows signiciant SNR difference between hemi-ring (SR3-IR3) and two sectors (SS4,SS5 – IS4,IS5), indicating superior depression of responses. The combination of SITA and mfVEP intersector analysis can confirm a suspecious gluacomatous visual field defect when it cannot be confirmed by a SAP test alone.

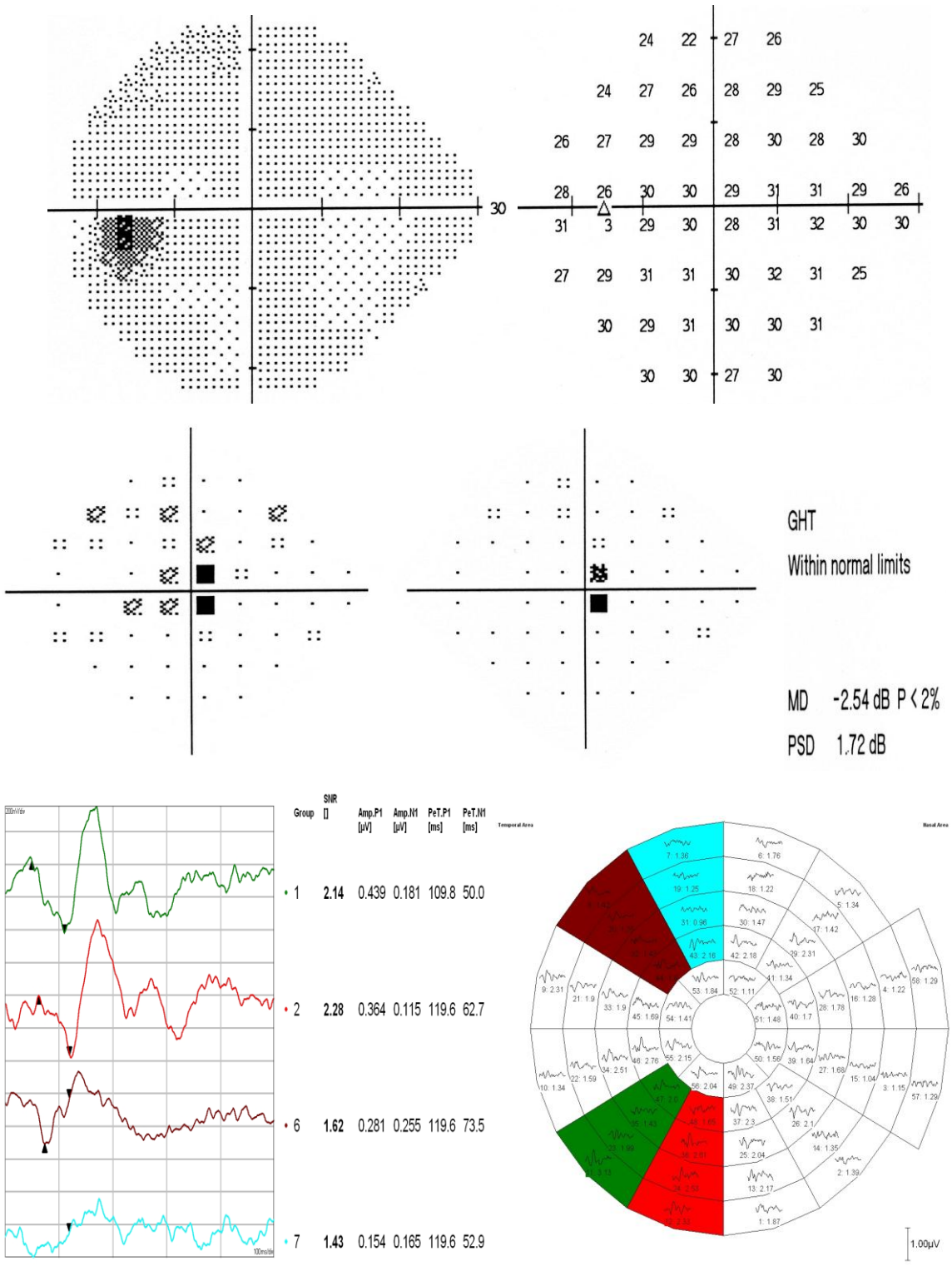



Figure (49) shows a suspicious SITA standard 24-2 test with superior central cluster of field defects shown in the total and pattern deviation plots. The GHT was “within normal limits”. mfVEP intersector hemifield analysis shows significant SNR difference between superior two sectors (SS4, SS5) and their corresponding ones in the inferior hemifield (IS4, IS5), indicating a possible superior depression of responses. The combination of SITA and mfVEP intersector analysis can confirm a suspicious gluacomatous visual field defect when it cannot be confirmed by a SAP test alone.

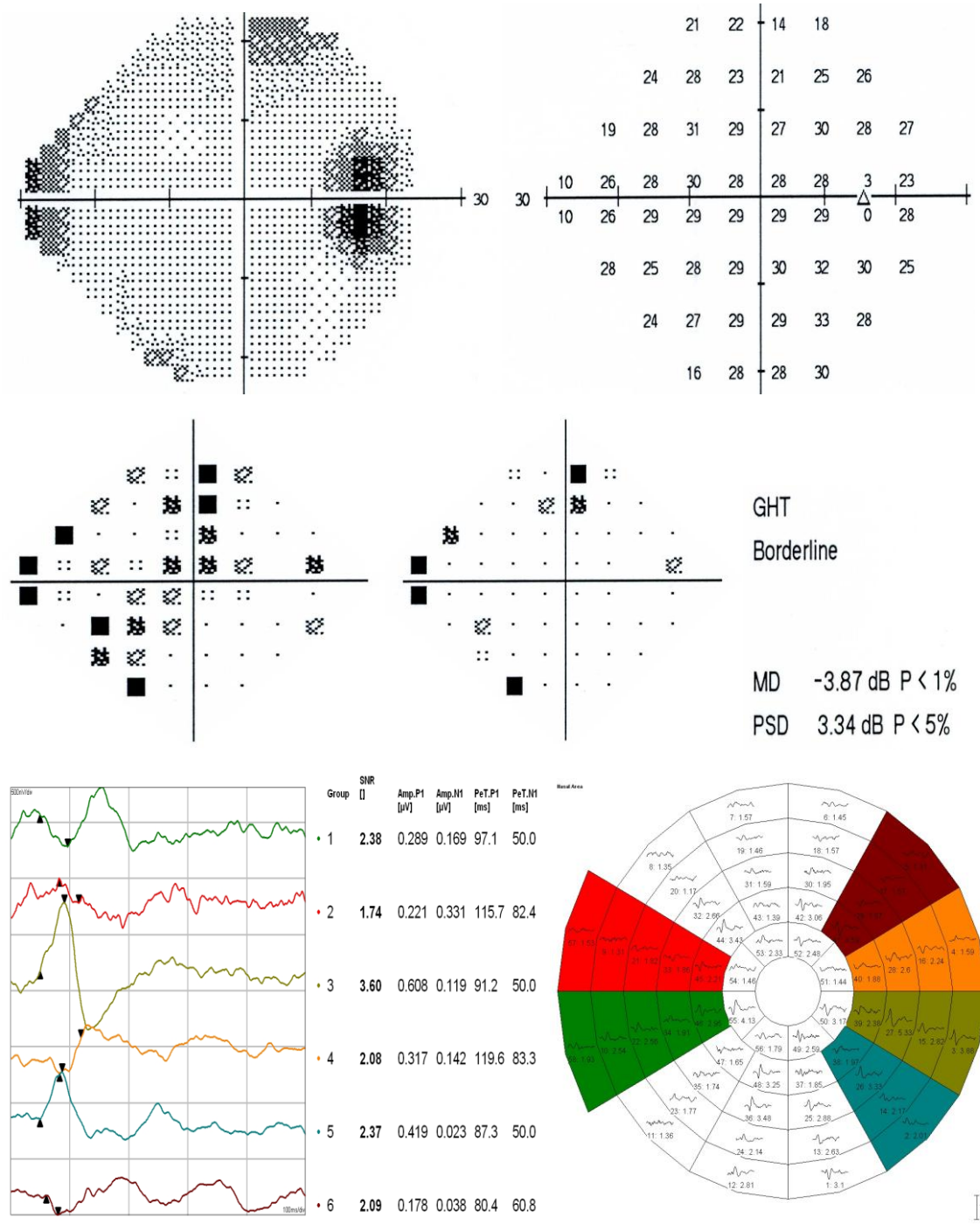



Figure (50) shows a suspicious SITA standard 24-2 test for a glaucoma suspect patient with scattered deep scotomas shown in total and pattern deviation plots. The GHT was “borderline”. mfVEP intersecotr analysis shows signifcant SNR difference between 3 superior sectors (SS1, SS5, SS6) numbered on the waveform plot (2, 6, 4 respectively) and their corresponding fellows in the inferior hemifield (IS1, IS5, IS6) numbered (1, 5, 3 respectively) indicating a possible superior response depression in these sectors. The combination of SITA and mfVEP intersector analysis is very helpful in these patients especially that SAP results are not conclusive and raising strong suspecion that glaucomatous visual field defect exists.

## 3.6.8 Examples of superior SITA test detection of visual field defects

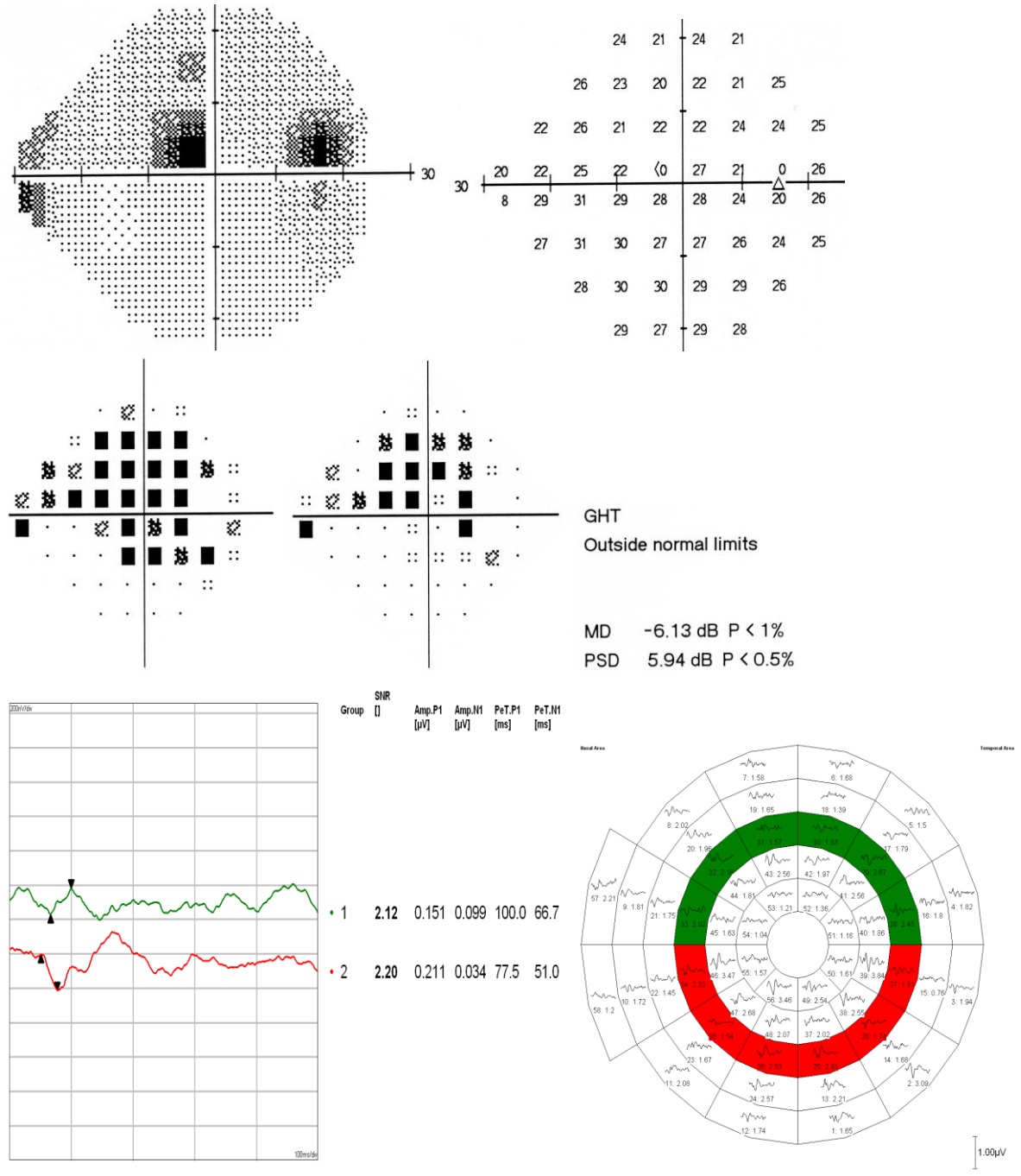



Figure (51) shows an example of a SITA standard test giving a better identification of glaucomatous visual field defects compared to mfVEP test. The superior deep arcuate defect shown in SITA printout is reproducible and consistent with the optic disc rim defect. On the other hand the hemi-ring analysis only shows moderate central ring depression in the superior hemifield compared to the corresponding inferior hemi-ring. The SITA test offered more informative and accurate representation of the glaucomatous field defect.

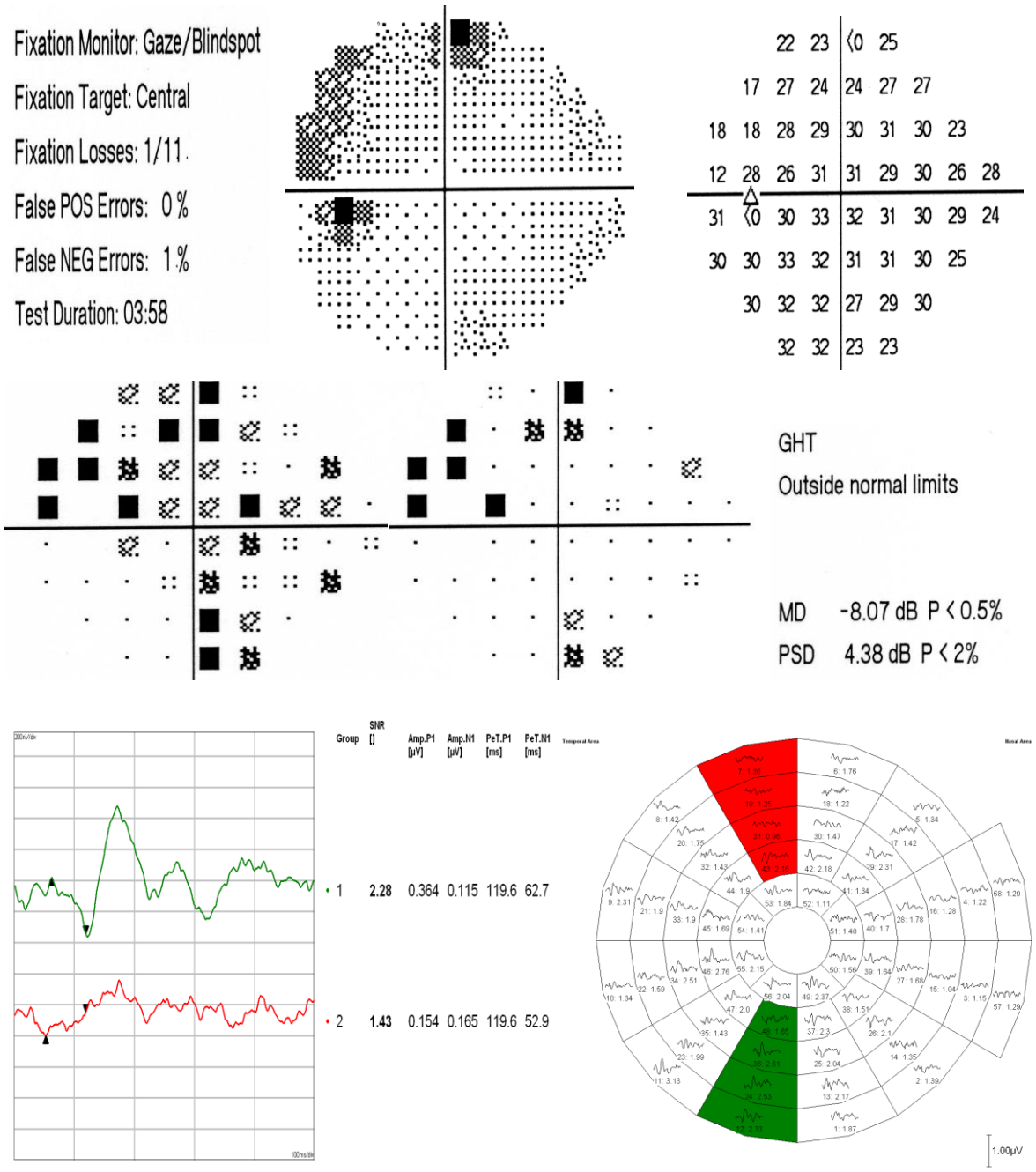



Figure (52) shows a SITA standard 24-2 test in a glaucoma patient. Both pattern and total deviation plots show signifcant glaucomatous deep superior arcuate defects matching the greytone printout. The test is reliable, and the defect is reproducible and consistent with the patient's clinical picture. mfVEP intersector analysis shows signifcant SNR value reduction in only one superior sector (SS4) compared to its corresponding sector in the inferior hemifield (IS4). The identified field defect in the mfVEP test is underestimated and smaller compared to how it is detected by the SITA test. Both tests confirm the presence of gluacomatous visual field defect in the superior hemifield. However, the mfVEP occasionally present the defect samller or disproportionate to its size shown by the SAP test, and most importantly, not matching the clinical picture of advanced cupping.

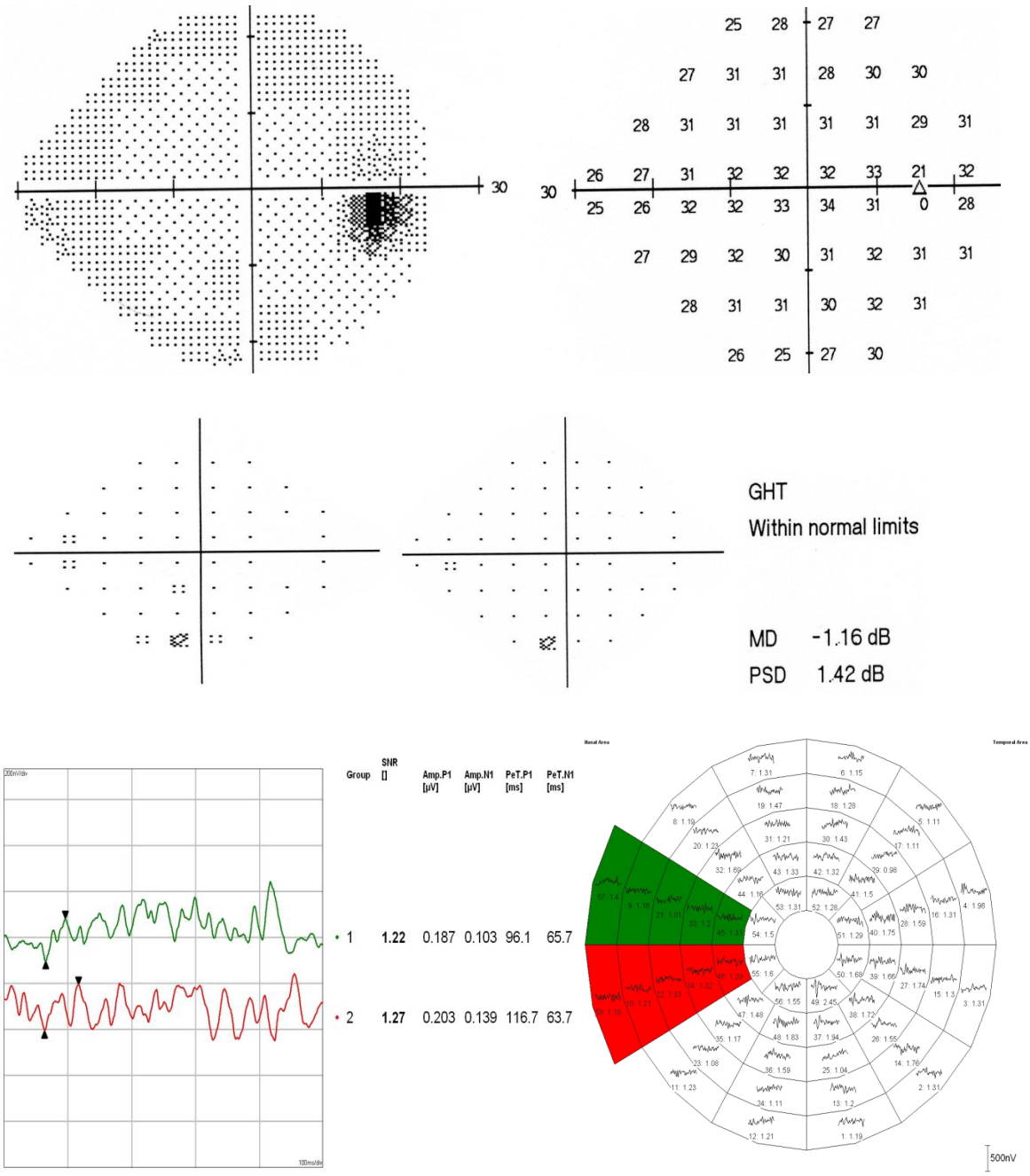



Figure (53) shows SITA standard 24-2 test with no significant field defects detected in a glaucoma suspect patient. These results were reproducible and consistent with the clinical picture of the optic disc. mfVEP Intersector analysis shows noisy waveforms with overall SNR depression in all sectors and hemi-rings. The two highlighted nasal sectors (red and green) show very low SNR (G5) without significant difference between the two hemifields. This poor SNR values most likely due to poor recording and/or connectivity errors, although it was the same after repeated attempts to reconnect all wires. Care should be paid when interpreting mfVEP results in absence of other data as results can be misleading. This example also highlights the need for good training and skill to interpret the results and discover errors carefully.

# 4.0 DISCUSSION

## 4.1 DEMOGRAPHIC DATA OF STUDY SUBJECTS

This study was performed on 3 different groups; one control of 20 normal subjects, glaucoma suspect group of 20 patients, and glaucoma group of 20 glaucoma patients. Each eye was considered as a separate entry in the intra-ocular analysis protocol of the mfVEP test. 112 eyes were included in this study, which was slightly greater than the calculated sample size to give a significance level of $P<0.05$. The results from this sample size are not strikingly different from previous studies carried out on the effectiveness of mfVEP in detecting glaucomatous visual field defects. A novel feature of this study is the assessment of the hemifield sector / hemi-ring analysis protocol as a new approach in mfVEP data analysis. The sample size is large enough to generalize some conclusions and observations on a larger population, considering the sample size included in similar studies, which ranged between 11 and 630 eyes [35,36,145,146,159,161,171,179-181]. However, further research work is needed to be performed on the assessment of this new analysis protocol to include the following: a longitudinal study to monitor repeatability of results, whether this protocol has any benefit in the early detection of glaucoma by monitoring defects detected over time, and to assess inter-test variability. The representation of the sample was equally distributed in number and age distribution amongst the three groups in order to eliminate any external factors that might influence the results. Normality tests were performed using standard Kolmogorov-Smirnov[a] and Shapiro-Wilk tests which confirmed a normal distribution pattern, and also using the Q-Q plot which provided a graphical presentation of the results. Parametric data analysis tests for all the data was carried out to check for signficance. As previously reported in this study and in previous studies [122,124,142,145,146]; many subjects found the mfVEP test to be easier, less challenging, less pressuring, more tolerable and perhaps more accurate than the standard HFA visual field test. Practically speaking, despite the lengthy testing time and inconvenience to some subjects due to the electrode connections and attentiveness required throughout the testing duration, the mfVEP test was appreciated by most of subjects as less challenging and does not require instant decisions like the HFA. Some patients, especially patients with an uncertain glaucoma diagnosis and glaucoma suspects, preferred the mfVEP test hoping that this new test could be more conclusive in their cases and give them a clearer diagnosis. The personal liking of many subjects has been stated in other studies [39,131,162], and perhaps could be an encouraging reason to clinicans and glaucoma practitioners to use the mfVEP in their clinic for their patients, especially when uncertainty and unreliability of HFA results exist. Most of glaucoma patients were experienced at SAP with a good learning curve. Normal subjects showed good cooperation in both tests and did good in both HFA tests, where sometimes it did not matter which test to be included in the analysis as both were accurate and reliable.

## 4.2 THE STANDARD AUTOMATED PERIMETRY RESULTS

Reliability of the SITA 24-2 test taken by the Humphrey field analyser was good in general; only 9 eyes showed reliability errors. Fixation loss was the most common error in glaucoma patients as expected and reported in previous studies [10,190-192]. Fixation is a problem exists in mfVEP as well, however, there is no dedicated tool within the test to check for test reliability objectively; still the current test configuration cannot provide a quantitative method to calculate a percentage of accuracy based on the subject's performance. This is one of the advantages of SAP, and a disadvantage of mfVEP test, which needs to be evaluated in future upgrading of testing software. False negative errors in the mfVEP test also artificially make the focal depressions worse, or deeper in terms of SAP measurements. Although the mfVEP does not depend on subjects responses or participation in assessing the visual field; subjects' inattentiveness, poor connectivity, and individual variability can give similar false negative errors. The two-run mfVEP test can check for intra-test variability, which is similar to fluctuation check in SAP tests. Theoretically, the intra-test variability can be automatically checked to exclude all widely variable values from certain segments if it is consistent. This software modification is suggested to be included in the hemifield sector analysis protocol. The global indices, mean deviation and pattern standard deviation, were as expected very low for normal group (1.08, 1.73 respectively). The values in the glaucoma group were significantly higher (-7.16 and 4.57). As also expected, the glaucoma suspect group showed average values of loss in mean deviation and PSD (-2.51 and 2.64 respectively). The grading system that used for glaucoma monitoring and assessment has too many inputs; some are clinical and others are related to reliable visual field test. These grading systems are complicated, and most often do not provide conclusive answers on progression of glaucoma. The benefit of the hemifield sector analysis protocol grading system is that it is easy to perform, depends objectively on SNR values of corresponding and fellow sectors, the comparison is automatically done and a colour coded signal can flag the location of possible signal depression, and finally it offers quantitative tools using measurable parameters to score and grade the visual field. This protocol provides guidelines to be utilized in a management plan from a clinical perspective, not just pure figures and numbers which can add more confusion to the decision making process. Practically speaking, the protocol still needs more evaluation and modifications based on the results of this study to make it less variable and control recording errors. There is a need for more objectivity in the diagnosis and monitoring of glaucoma.

## 4.3 PERFORMANCE OF SAP VISUAL FIELD TEST

In this study, it was detected that certain subjects showed better assessment of their visual field with SAP. Figures 51-53 show examples of SAP yielding a more reliable and consistent visual field assessment compared to the hemifield sector analysis of mfVEP test. In these subjects, the clinical picture and the consistency of the defects detected by the SAP visual field test confirmed its accuracy, while mfVEP did not show or estimate the defect accurately. In cases shown in figure (51) and (52) the hemifield sector analysis protocol underestimated the glaucomatous visual field defect, made it smaller in size (less sectors or hemi-rings), shallower depression (SNR value averaged around 2.2) which is not as deep as it was shown in the SAP results, and the SNR difference values between the two hemifields were not that big although there was a significant difference between the two opposite hemifields shown in the SAP results such as subject in figure 51. The subject shown in figure (53) had significant noisy recordings with poor SNR values, which may explain why the glaucomatous defect was not shown properly in the mfVEP, with significant false positive values. There is no clear explanation for the findings on figure 51 & 53, as why mfVEP test did not detect these defects accurately. Some possible explanation is related to the subjects' unique anatomical landmarks that could have underestimated the true signal and give rise to false negative recordings. This explanation is supported by previous work explained by Hood et al [(39)] who suggested that occasional misdetection of glaucomatous visual field defects can occur due to the orientation of V1 visual cortex to the bipolar electrodes. They concluded that in the majority of the time the mfVEP could detect glaucomatous visual field defects earlier than SAP. However, the reverse can occur as well, assuming that SAP results are reliable. Their analysis suggested that the two tests will often agree. Nevertheless, regardless of the accuracy of mfVEP results and its ability to detect visual field defects earlier, the SAP cannot be replaced by any other test. Another possible explanation is that SAP visual field testing can be exceptionally better in certain patients who perform the test at high reliability manner. Certain patients are good and reliable SAP test takers and poor mfVEP takers for no obvious reasons.

## 4.4 MULTIFOCAL VEP RESULTS

### 4.4.1 Main mfVEP test parameters

The results of the multifocal VEP testing showed 3 main types of outcomes; the ability of the test to confirm already existing glaucomatous visual field defects, the ability to confirm normal visual

field in normal subjects, and the role in the early detection of glaucomatous visual field defects in suspicious and suspect patients. A novel analysis protocol was used in this work where intra-ocular comparison of corresponding hemifield sectors and hemi-rings were analysed. The hemifield sector / hemi-ring analysis showed promising results in the three main outcomes when used to analyse the mfVEP tests in different subjects. In all the analyses two main parameters were used; firstly, the SNR values of individual segments, and the averaged SNR values of sectors and hemi-rings and secondly , the intersector and hemi-ring SNR difference (SNR difference value), which is the difference between each pair of corresponding sectors and hemi-rings in the two opposite hemifields within each eye. Two types of comparisons were made.

1) The intra-group comparison, which is the direct SNR comparison between sectors across the midline looking for any significant difference points to focal sector or hemi-ring depression as a representation of visual field defect.
2) The Inter-group comparison, which is the comparison between the three groups. This type of comparison involved two sub-types:
    a- The direct comparison of sector and hemi-ring SNR values in similar locations among the three groups, which were examined for any significant differences between similar sectors and hemi-rings between normal, as the control reference, and the glaucoma and glaucoma suspect groups.
    b- The SNR difference values between the two hemifields in each eye were compared as a standalone parameter (SNR hemifield difference) between the three groups, examining for any significant difference between the three groups points to a theoretical hypothesis that glaucomatous eyes should have big SNR difference between the two hemifields when compared to normal subjects, where the two hemifields are almost identical.

## 4.4.2 mfVEP intra-group analysis

### 4.4.2.1 Normal group

As per the inclusion criteria; a normal subject did not have any ocular disease that could affect their field of vision directly or indirectly through medications or other influences. In the intra-group analysis, when the inter-sector/hemi-ring differences between corresponding hemifields were tested for significance, a comparison between the 6 sectors and 5 hemi-rings on each hemifield was carried out. Only one pair of corresponding sectors was statistically signifcant (SS5 -

IS5). This result was concordant with the hypothesis but also logical thinking, as normal subjects should not have any signifcant difference between the two hemifields. Only one sector yielded a statistically signifcant difference between the two hemifields, which could be attributed to the wide normal range of normal SNR responses among the normal group. Normal SNR response in this study showed a mean of 2.85. However the variability was the highest amongst the 3 groups (SD = 0.503), which could be expalined in the light of normal distribution of the data, as a normal variation. Many studies have shown evidence of a wide range of SNRs among normal individuals [39,71,131-134]. The variability in the normal subjects showed a mean SNR varied by a factor of 3, from about 2.0–6.0 in many studies [132-136]. The relatively small SNRs in a few individuals can be traced to high noise levels. Most of these variations were attributed to a variation in the size of the signals. It is this range of SNR values that creates a problem for tests of significance, especially those based upon analyses of monocular mfVEP test. Variability issues even among normal individuals pushed researchers into one of two directions to overcome this problem and make their data valid. [136]. Moreover, in similar studies of monocular mfVEP testing the SNR values obtained from individual segments showed 7 or even 10 folds (SNR average 9.5) which is close to the values obtained and recorded for normal subjects in this study, which ranged between (2.78 and 13.8) throughout all 58 segments, and of course following the cotrical representation rule of higher values more centrally and lower towards the periphery. A difference in this study is that averaging of the SNR values was used from a specific group of segments representing a sector or a hemi-ring, and in this case the SNR value is less and more consistent all over the entire field with very minimal variation between peripheral and central sectors and hemi-rings. Each pair of sectors and hemi-rings had paired t-test performed to check for statistical significance. The major problem with running a mfVEP monocular test is the need to reference values to establish a comparison, which is why it was stated that the variability of mfVEP among normal individuals necessitates the need for establishing a normative database for the test with accepted range of standard deviation from the mean values for central and peripheral responses of the visual field. The variability of mfVEP among normal individuals made it necessary for researchers to standardize a suitable normative database that could be used for patients with an accepted range of standard deviation from the mean values for central and peripheral responses of the visual field. However, this normative data cannot be standardized as a universal database in any testing equipment, but many commercially available software packages use some form of normative database in their testing procedure to produce grading scale with probability maps; VERIS™ and AccuMap® systems are examples of these software. The most suitable method suggested by many authors [19,39,159,160] is for each clinic to create its own age-related normative data. The advantage of the hemifield

sector analysis protocol is that it provides an intrinsic method of standardization and referencing of SNR values by comparing within the same eye corresponding sectors and hemi-rings, which does not require any external normative data in identifying the difference between normal and glaucomatous focal visual field defects. In addition, what determines the usefulness of a test that measures the progression in glaucomatous visual field defects is its good repeatability. mfVEP has shown good repeatability in many studies, even better than SAP protocols [142,145,146,161,162].

#### 4.4.2.2 Glaucoma suspect group

Glaucoma suspects present uncertainty and confusion to many clinicians when reaching a diagnosis, especially as the main tool for visual assessment is the SAP visual field testing. This group of patients usually presents with one or more positive clinical findings or risk factors for glaucoma, but without definite optic neuropathy or established glaucomatous visual field loss. In this study group, there was a lack of homogeneity in the clinical findings and level of expected defects among patients. Some patients had repeated abnormal visual fields with focal defects that do not match a healthy disc appearance, but high intraocular pressure was reported. In other patients they were considered glaucoma suspects on the basis of a suspicious optic disc appearance, disparity in cup-to-disc ratio between the eyes, persistently high intraocular pressure, and positive family history, but in the absence of significant visual field defects. The lack of homogeneity can be attributed to the nature of the diagnosis, and how it is related to many variable findings and clinical signs that each one of them can be considered a reason to suspect glaucoma. However, the presence of significant visual field defects could have moved those patients into the category of glaucoma patients, since persistent and reproducible visual field defects in the absence of other findings could be viewed as an early functional deficit. If the visual field assessment is not accurate or equivocal then the diagnosis of glaucoma could be missed or the treatment may commence without conclusive evidence that a glaucomatous process is ongoing. Using the sector hemifield analysis protocol, it was found that 4/6 sectors showed a statistically signifcant SNR difference when compared to their corresponding fellows, while 1/5 hemi-rings was statistically significant. This result is expected based on the nature of diagnosis in this group, as most of the SNR values were very close to normal range, but distinctly far from the glaucoma SNR range of values. The presence of this signifcant difference between the two opposite hemifields could give an indication that there are focal depressions in the responses in certain parts of the visual field, and the mfVEP was able to detect the focal depressions and their location. Most of the glaucoma suspect patients enrolled in this study presented with high

intraocular pressure as a major risk factor for glaucoma. These results are not conclusive, nor can they confirm that the patients found to have a statistically significant difference between the responses in the two opposite hemifields, have an early process of glaucoma taking place. There is a lack of longitudinal data in this research to establish whether these patients will develop glaucoma in the future. This limitation necessitates the need for longitudinal study. However, numerous studies have reported that mfVEP can detect early glaucomatous field defects by a significant reduction in the SNR response, or ampitude values. Recent evidence suggests that mfVEP can have clear role in monitoring and detecting progression of glaucoma based on good repeatability figures [19,145,159,170,171]. There were many examples where HFA and mfVEP were not consistent and did not agree in the detection of visual field defects. These results are in agreement with Chen [145], Goldberg [134] and Greenstein [172] who showed that mfVEP detected damage missed by HFA. Furthermore, abnormal mfVEPs have been reported in patients with normal visual fields assessed with the HFA. These findings have been attributed to the subjective responses of the patient in SAP and the learning curve that can mask shallow and early defects. Obviously, there is no learning curve required for mfVEP test, which can make the detection of small visual field changes more reliable. However, it is important to emphasize that in order to perform a mfVEP test accurately, both the operator and interpreter of the test should have a minimum level of technical skills and sufficient knowledge of the test process. This requirement can be considered as a form of "learning curve", which can influence the reliability and accuracy of the test, similar to that of the SAP test.

#### 4.4.2.3 Glaucoma group

The mfVEP was extensively studied in the assessment of glaucomatous visual field defects and was found to have excellent results, yielding high sensitivity and specificity [39,73,120,123,174,176,225]. In this study, the majority of patients suffered from chronic open angle glaucoma, which is the most common type of glaucoma worldwide [6,7]. Recruitment was designed to give a representative sample of patients with variable degrees of severity represented by their visual field defects. This sample presented with defects ranging between early focal scotomas to the deep and extensive visual field losses which are a feature of advanced glaucoma. Most of the patients (13/20) had asymmetrical glaucomatous field changes between eyes, the rest (7/20) had symmetrical mild to moderate field changes defined by the Hodapp – Anderson classification [216,219] . The results show that the majority of patients had asymmetrical glaucomatous visual field defects between the two eyes, which is a good confirmation of how important the monocular mfVEP test analysis is. The

monocular analysis is more beneficial in these patients simply because for inter-ocular analysis, it is assumed that one eye is a “normal” reference for the glaucomatous one and therefore it will not detect the defects accurately. The presence of symmetrical defects usually influences the ability of inter-ocular tests to idenfity defects. This has been confirmed by many studies, thus the inter-ocular comparison test might not detect bilateral damage located in corresponding field locations [39,137]. The mean SNR value for glaucoma group was 1.70 ± 0.412, which is low average response, close to poor (SNR = 1). This SNR average confirms the ability of the HSA protocol to identify glaucomatous visual field defects accurately. Only 8/36 eyes exhibited advanced glaucomatous changes with symmetrical severe field loss across the midline in both hemifields. The remainder of the sample (28/36) showed clear differences between the two corresponding hemifields. In such patients, the hemifield sector analysis protocol used in this research will easily detect isolated focal defects in one hemifield sector or hemi-ring by direct comparison to their fellows in the opposite hemifield. The results confirmed this hypothesis, and it was found by running the inter-sector/hemi-ring differences between corresponding hemifields in 6 pairs of sectors and 5 hemi-rings on each hemifield that all sectors and hemi-rings were statistically signifcant when compared to their coresponding fellows in the opposite hemifield. This means that regardless of the level or severity of the glaucomatous visual field defect, the hemifield sector analysis protocol of mfVEP was able to identify the defect and its location and highlight a low response due to the field defect. These results also in agreement of several studies carried out over the past two decades, where the ability of mfVEP test to localize and identify already established glaucomatous visual field defects was confirmed [39,125,133,142,144,146,174,178,225].

### 4.4.3 mfVEP Inter-group Analysis

For the inter-group analysis, with its two sub-analyses (the sector SNR values and the intersector / hemi-ring difference analysis) there was a statistically signifcant difference in sectors and hemi-ring SNR values between the 3 groups. This result is no exception from the initial hypothesis in that for normal subjects SNR responses will be of a higher quality and much larger in amplitude and shape compared to the glaucomatous patients’ visual field. Every sector and hemi-ring showed a statistically signifcant difference between the 3 groups, indicating that the normal averaged SNR values of sectors and hemi-rings varied signifcantly from both glaucoma and glaucoma suspect groups (SNR; 2.85, 1.70 and 2.27 respectively). These results were confirmed in previous work [131-135] where low SNR values were recorded from different parts of glaucomatous visual field

following the classic distribution of visual field losses in glaucoma. The SNR difference values were also compared in the 3 groups. The differences in values between corresponding sectors and hemi-rings across the midline were statistically signifcant between the 3 groups. This means that each individual comparison between allocated sectors and hemi-rings in the two opposite hemifields was statistically signifcant between normal, glaucoma and glaucoma suspect groups (mean SNR difference values; 0.28, 0.36 and 0.22 respectively). It was noted that the mean SNR difference values of glaucoma suspects was apparently not much lower than normal controls, which could imply that the SNR values in glaucoma suspect group showed higher quality values. This could be explained by the larger variability of the normal group compared to a well specified and selected group of glaucoma suspect patients. Theoretically and from a clinical viewpoint, the normal controls are closer in values to glaucoma suspect patients, as these patients do not have by definition true visual field loss or glaucoamtous optic neuropathy. However, there is greater homogeneity within the glaucoma suspect group with lower variability (SD = 0.184) compared to the normal controls (SD = 0.223). The same is true in the averaged SNR values of each sector and hemi-ring, where variability in normal group was higher (SD = 0.503) compared to the glaucoma suspect group (SD = 0.276).

The Inter-group analysis yielded a statistically significant difference between the 3 groups in both sub-analyses; the averaged sector and hemi-ring SNR values, and the intersector SNR difference values. The SNR values in glaucoma group was low compared to the normal controls (SNR 1.70 and 2.85 respectively). The SNR difference values between the two opposite hemifield were also statistically significant in the glaucoma group compared to normal controls (SNR difference 0.36, 0.28 respectively). The large intersector difference in the glaucoma group indicates and matches the underlying glaucomatous visual field defect, which was more clearly identified when each hemifield was compared to its opposite fellow. When this was compared with the normal group where the difference is naturally smaller, it was concluded that this protocol can confirm the existence of already established glaucoamtous defects by two different ways; firstly by the low value of the SNR, and secondly by the larger SNR difference between the two corresponding hemifield sectors and hemi-rings.

The SNR difference values in individual sectors and hemi-rings were examined to evaluate the most common locations found to be statistically significant. Figure (26) shows that 4/6 sectors were statsitcally significant, mainly taking the classic arcuate pattern of glaucomatous visual field defects. It was also found that 3/5 hemi-rings (the innermost hemi-rings) were statistically significant. This study cannot provide sufficient explanation for the specific locations which were

found to be statistically significant between groups. However, these locations most likely related to the unique distribution of visual field defects characteristic of this group of patients, and it could alter in a different group of patients. Theoretically, there is no explanation which supports the opinion that these locations are more liable or easier to detect using this protocol.

Figure (37) shows that some defects were detected by the hemi-ring configuration analysis while missed by the sector analysis, and vice versa, sectors can detect some missed defects by the hemi-ring analysis. This could be explained by the anatomical nature of the defect. This finding dictates the need to carry out both analysis protocols for every mfVEP in order not to miss a focal defect.

## 4.5 ACCURACY OF MFVEP

### 4.5.1 mfVEP Sensitivity and Specificity

The sensitivity and specificity of the hemifield sector analysis protocol of mfVEP test in detecting glaucoma suspects were in this study 89% and 79% respectively. These figures are consistent with many previous study suggested a role of mfVEP objective test in the early detection of glaucoma. The majority of studies identifying the role of the mfVEP in the detection of glaucomatous visual field defects confirmed its ability to detect defects that are not detected by SAP with good sensitivity and specificity levels (76%-92%), which is a very good detection rate compared to the SAP results and its limitations in the early stages of glaucoma. A larger scale study would establish whether these figures translate into a larger population. The sensitivity and specificity of the hemifield sector analysis protocol in detecting glaucomatous defects were 97% and 86% respectively. These figures are supported by previous studies where the majority of studies identifying the role of mfVEP in detection of glaucomatous visual field defects confirmed its ability to detect already existent damage with high sensitivities ranging between (86%-97.5%) which is a very good detection rate when compared to SAP and its limitations in the early stages of glaucoma [145,159,161,171,179-181].

### 4.5.2 Repeatability and Variability

The ultimate goal of any new test designed to diagnose and monitor glaucomatous progression is to have good repeatability and can offer monitoring options with a high degree of accuracy. A

number of approaches have been used in identifying glaucomatous progression and monitoring visual function; these include the clinical judgment, using different classification and scoring systems for visual field defects such as the AGIS and the collaborative initial glaucoma treatment (CIGTS). Although the SAP global indices are used extensively in research to use it as an indicator of progression [10,16,17,30,190-192,212], it is still evident from the literature that no agreement exists on the best method for differentiating whether a glaucomatous visual field defect is stable or progressing. The mfVEP has shown very promising results regarding monitoring and follow up of glaucoma defects with good sensitivity and specificity figures. This study did not evaluate repeatability of the mfVEP test and how much the hemifield sector analysis results would change with inter-test variability. However, the mfVEP test has shown in many studies a good level of repeatability among normal subjects, and glaucoma patients, in short and long reassessment intervals. Klistorner et al [146] evaluated the mfVEP responses in controls and glaucoma patients on two separate occasions and they found no significant differences in the responses across the field. Later, Klistorner et al [196] measured the coefficient of variation between two successive mfVEP tests in two groups, and reported a low value (16%) confirming a good level of repeatability of the mfVEP test. Other studies compared the repeatability of the mfVEP tests compared to the standard HFA results, such as Chen et al [145] who compared the repeat reliability of both mfVEP and HFA tests in a retrospective study and reported a better repeatability of the mfVEP test, where the signal amplitudes were more reliable than the SAP thresholds. More recently, Fortune et al [119] evaluated the repeatability of the mfVEP test and compared it with the SAP repeatability parameters in a group of normal subjects in two separate occasions one year apart. They found that the repeatability of mfVEP was slightly better than SAP visual field results after one year of retest. Based on their findings they advocated the use of mfVEP in the early progression stages of glaucoma as it would be much easier to detect glaucomatous visual field changes. They also reported some limitation in the peripheral locations of mfVEP test, the low dynamic range of SNR calculations made the detection of progression in these locations more difficult and unreliable. One major problem of the current mfVEP settings is its narrow dynamic range which may limit the detection of the deepening of an already established visual field defect, especially in the advanced glaucoma stage [39,161]. A deep scotoma or visual field defect is something not defined by the current mfVEP testing protocol. Nevertheless, it can detect accurately the location and size in a poorly designed spatial resolution, but only in terms of SNR values and amplitude differences. These values do not have a categorical system or logarithmic scale that can be used to measure increase or decrease in its values over time. This problem significantly influences the ability of mfVEP to be used in monitoring glaucoma, even when its sensitivity is high and variability is less.

Another limiting factor for the mfVEP use in monitoring glaucoma is that monocular testing does not provide any useful information by itself to the clinicians and glaucoma management team unless its results compared to reference values. For this reason, the hemifield sector analysis could be a possible method to overcome this problem, as it uses the patient's own data as reference in close similarity.

## 4.6 SUPERIOR PERFORMANCE OF MFVEP

The mfVEP has been used in clinical research on patients with various types of glaucoma to evaluate the glaucomatous functional loss objectively. Their results confirmed the ability and credibility of mfVEP test to detect glaucomatous visual field loss. Whether mfVEP was assessed alone to detect visual field defects or in comparison to HFA, it has been always able to detect glaucomatous defects. In this study the two tests were used and their results were compared to each other in the three groups. The hemifield sector analysis protocol applied to the mfVEP test showed the ability to detect glaucomatous visual field defects confirmed by the HFA tests. In addition, mfVEP was also able to confirm normal responses in normal subjects. Most of the differences between the two tests were clearly shown in the glaucoma suspect group and the suspicious HFA results, where mfVEP added important information about the assessment of these subjects' visual field.

### 4.6.1 Confirming normal visual field results

The hemifield sector analysis was performed on all normal subjects included in the control group. When sectors and hemi-rings were compared across the field all normal subjects were confirmed to have normal visual field. No single mfVEP test was reported as abnormal or suspicious in normal subject group. Figures 34 & 35 are examples of normal subjects' recordings. The SITA standard test was also normal in those subjects. In addition, the hemifield sector analysis was also normal and showed no significant difference between the two hemifields, confirming their normal status. This finding is crucially important, as confirming normal visual field is as important as confirming glaucomatous visual field defects. Most of the previous research work confirmed the ability of mfVEP tests to detect glaucomatous visual field defects but without the important comment on its ability to confirm a normal field. In the presence of good signals and analysis evaluated by SNR

values instead of the amplitude or latency analysis, the outcome is better and increases the reliability of the results.

### 4.6.2 Confirming glaucomatous visual field defects

As expected and mentioned in the initial hypothesis, mfVEP was able to detect already established glaucomatous visual field defects in glaucoma patients. All patients with variable degrees and severity levels of glaucoma were assessed using the hemifield sector analysis protocol and their results confirmed the defects. Interestingly, even in patients with advanced glaucomatous defects, where both hemifields are damaged, probably equal, the hemifield analysis protocol confirmed the defect indirectly by the generalized low SNR values on both hemifields. The advantage of this protocol is that it can detect the defect if significant SNR difference between the two hemifields is recorded, and in addition, it can prompt the clinician to a state of generalized depression in SNR values if advanced glaucomatous visual field defects are present. Figure 35 shows that clearly. In the top recording, the significant difference between each pair of corresponding sectors pointed to the existence of focal glaucomatous visual field defect. While in the bottom recording the advanced glaucomatous defect in both hemifields did not give any significant difference between corresponding sectors and hemi-rings, but instead it showed generalized depression in all sectors and hemi-rings across the midline. Figures 38-42 show examples of glaucomatous visual field defects of variable severity and grades. The hemifield sector analysis was performed in all subjects and confirmed the focal defects, regardless of its size or location; it was detected by the direct comparison or by the symmetrical SNR depression in one sector or more of the visual field. These results are in agreement with many studies. For example, Goldberg [(134)] used the mfVEP test to detect glaucomatous visual field defects already documented in glaucoma patients. He found good correlation with the HFA field results in glaucoma patients, and reported that mfVEP can assess the visual field and identifies glaucomatous visual field defects, and that it may have the potential for identifying defects earlier than conventional perimetry. Graham et al [(159)] also reported similar results, and showed evidence that mfVEP is an effective method for detecting visual field loss in glaucoma. They suggested that the mfVEP test provides a valuable aid to the clinician in categorizing patients with unreliable, variable, unconfirmed, or excessive subjective field loss.

### 4.6.3 Early detection of glaucomatous visual field defects

The role of mfVEP in the early detection of glaucomatous visual field defects, and in confirming an unreliable visual field test is considered the main area of recent research. Recent evidence suggests that the mfVEP can have clear role in monitoring and detecting progression of glaucoma based on good repeatability figures [19,159,170,171]. Glaucoma suspect patients and subjects with suspicious visual field tests are usually the main source of confusion and uncertainty in glaucoma practice. Most of glaucoma specialists depend in their day-to-day practice on the SAP techniques to diagnose and monitor their glaucoma patients, and when SAP results are unreliable or inconclusive it reflects on the management plan and the follow up visits. In this study, the hemifield sector analysis protocol was able to provide interesting results in some patients with glaucoma suspect diagnosis or patients with unreliable SAP results. Figures 43-47 are examples of glaucoma suspect patient with inconclusive visual field results. The mfVEP test results showed in almost all of them confirmed significant focal depression in one or two sectors. The focal depression detection usually reflects glaucomatous visual field defect, as the SNR value represent the response of each segment, and theoretically if the response in consistently low or depressed this should be a good indicator that there is a focal defect in this location. Modest visual field losses lead to very small mfVEP responses. Thus, the presence of a good SNR indicates that the visual field should be relatively good. This observation has an important clinical implication. Monocular field analysis measures focal defects precisely, the interpretation of these defects and how reliable it is could be answered by comparing the results of the two tests. Most of the cases shown in these figures did not show significant SAP changes or defects, in fact the GHT was within normal limits. While there was a good reason for those patients to be flagged as suspects; either by high intraocular pressure or family history, the presence of additional information from the mfVEP test should alert the clinician to modify his management plan or give him an indication to closely monitor this patient as there might be an early ongoing process of glaucomatous damage. Up until this point, there has been no conclusive role of the mfVEP as a solid parameter confirming a visual field defect due to glaucoma. Most likely its role can be as an adjunct to existing data, and may be used to aid the doctor when making a decision whether early glaucomatous damage may be expected. One exception is shown in the case presented in figure 46, showing a glaucoma patient on treatment, where the size and depth of visual field defect shown in the SAP test result did not match the clinical picture of this patient, as he should have deeper and larger defect. The mfVEP sector analysis protocol showed a much more extensive defect involving more sectors and the SNR values markedly reduced which could give an indication of deeper glaucomatous visual field defect. However, these mfVEP tests need to be repeatable and the defects are accurately

reproducible in order to finalize the diagnosis. Long term follow up of this patient would reveal if the mfVEP is predictive of future SAP visual field loss.

## 4.7 CURRENT AND FUTURE ROLE OF SAP TESTS IN ASSESSMENT OF VISUAL FIELD

In this study, it was found that the hemifield sector analysis protocol of mfVEP test provided a sharp demarcation between a normal visual field and a visual field with glaucomatous defects. This conclusion was based on a single examination comparing its results to the gold standard HFA visual field test. Even within the suspicious and glaucoma suspect group, mfVEP was able to provide additional important information, yet to be confirmed longitudinally, but worth following and assessed in future studies. However, many previous studies proved the superiority of mfVEP in sensitivity, reliability and repeatability [39,134,196,198] which suggests that the introduction of mfVEP testing in glaucoma clinics would be a very useful addition as it may enhance diagnosis in suspicious cases. The hemifield sector analysis offers a simple method even for inexperienced operators who are not involved deeply in assessing mfVEP row data, and a direct way of judging the visual field and highlight possible focal depressions. These findings would support the need for the use of mfVEP visual field assessment for monitoring glaucoma patients as well, with a good repeatability and lower variability rates; this test has the potential to take its expected place. On the other hand, the question whether SAP visual field assessment is still needed clinically seems to be answered, as SAP still the best (and most practical) test available in the day-to-day assessment of visual field.

Many researchers and glaucoma experts believe that no alternative visual field test available at this time can totally replace the SAP test. This is for the following reasons:

1- Almost all sources of SAP unreliability, except for learning curve, related to the subject or the test procedure can be found in mfVEP as well; including but not limited to media opacity, eye lid problems, exhaustion and inattentiveness, fixation and lengthy test duration
2- There is no single test can give information about the visual field with the same spatial resolution as SAP. The spatial resolution of mfVEP is loose; the sectors at the periphery are in some locations over $7^{o}$ in width, leaving large spaces in the peripheral field as untested. In this case it is difficult to establish a diagnosis as a confirmed visual field defect

considering only one response from such a large area, two or three adjacent points with individual responses would have been more confirmatory as proved before in many studies [71,126,134,161,202].

3- The mfVEP requires special training and basic knowledge about electrophysiology concepts. In addition, there are many sources of connection and electrode montage errors that can easily give rise to false positives, or occasionally false negatives if fixation is not monitored.
4- In SAP there is now a complex normative and glaucomatous dataset which has been accumulated over decades of research work playing a solid role in the favour of conventional SAP testing. The mfVEP data does not have reference normative data, on the contrary population based normative data is useless with mfVEP due to individual anatomical variations, which can create great deal of variability if population based data used as a reference, and in this case it will be extremely difficult to define what is normal and what is pathological.
5- Despite that it was reported in this study and elsewhere [39,146,161,167] that patients preferred the mfVEP test over SAP visual field assessment for variable reasons, the test from practical point of view and from an outpatient setup perspective, still considered time consuming and inconvenient for both patients and clinic flow. SAP is quicker to administer with no electrode mounting or experienced technical staff is appreciated by clinicians much better than mfVEP.

mfVEP should not be advertised or promoted as a substitute of SAP visual assessment, neither do the results of this study nor previous work can suggest such a promotion. The mfVEP has shown very promising results as a supplementary important test that can be used in certain patients as a source of additional objective information for specific category of subjects.

## 4.8 AGREEMENT BETWEEN SAP AND MFVEP

This work could not establish any correlation between depth of visual field defect on the HFA and the SNR values. The defect depth is determined by two main visual field parameters; the mean deviation and pattern standard deviation, each using a different method of calculation from the SNR values. In order to establish a direct correlation measure of the two tests, a probability map could be made for both tests but is not very accurate in the case of mfVEP as proved by previous work [39,134,142]. The SNR of monocular responses can be used in situations where the clinician

suspects that a large visual field defect is not ‘‘real’’. In his results, Hood et al [39] has shown that the presence of good SNR value almost always indicates that the visual field in this specific region is good as well. But conversely, a small mfVEP response does not necessarily mean that there will be a visual field defect or that there is a confirmed glaucomatous damage in this location, despite a large response means that the visual field sensitivity should be reasonably good, and If it is not, the SAP visual field should be questioned because a repeatable and confirmed mfVEP response is more sensitive than SAP test results. Monocular field analysis measures focal defects precisely, the interpretation of these defects and how reliable it is could be answered by comparing the results of the two tests. These measures are comparable in that it assesses the same region (the sectors of the mfVEP display versus the SAP retinal sensitivity map) of the visual field. However, the two measures do not have a comparable ratio scales; there is no direct relationship between decibels and SNR values. In addition, they do not possess the same spatial resolution. In this study the results of the two tests were evaluated indirectly by comparing the two grading systems; the HFA visual field grading system and a simple grading system which was created in this study categorizing SNR values into 5 levels running between normal (good) and pure noise. In this study kappa analysis was used for agreement between the two tests using the grading system of both tests. The agreement was 88.9% in identifying normal subjects and 77.8% in identifying glaucoma patients. The agreement was higher for identifying normal subjects since all normal subjects were reported as normal in both tests, which is an expected finding due to the higher SNR value, insignificant SNR difference values, and the better quality of mfVEP recording. Glaucoma patients exhibited lower agreement because of low responses and lower reliability in both tests. These findings are in agreement with previous studies, where SAP and mfVEP results were comparable and showed similar repeatability figures [134,142,145,146,198].

## 4.9 COMPARISON OF HFA AND SNR GRADING SYSTEMS

Both mfVEP and SAP test strategies test the same visual field using different techniques and probability maps. These techniques and more importantly the probability maps, do not match in their spatial resolution or in the interpretation of their meanings. The tested visual field is sampled in a very different way by the two techniques. Within the central 2.61 degrees (5.21 degrees diameter) there are 12 mfVEP responses covering this location, while only one HFA test point is covering this area in the standard 24-2 protocol. Conversely, in the outer testing rings, three or four HFA test points fall within each of the individual segments. The conclusion is that for two differently scaled tests spatially it is difficult to be sure which points can be corresponding to

its similar region in the other test. There is clearly a large range of amplitudes for the normal controls [39,76,77,133,145,159,199,200]. This normal range includes very low amplitude responses in some locations, but larger amplitudes in other locations, which do not reflect the same retinal sensitivity distribution seen in SAP results [77,200]. As a result of this mismatch, it is sometimes difficult to distinguish what is normal from abnormality using a single test, especially clearer in the case of mfVEP. The idea of comparing the results of the two tests is sound and valid, and definitely will add new information about the assessment of the visual field, assuming that the two tests are compared accurately, considering the important differences between them in spatial resolution. In this study the similarities and variations of the two field grading systems are illustrated in a graphical way to highlight a few important points. Figure (19) shows the three groups classified according to the SNR grading system. Similarly, figure (20) shows the three groups classified according to the HFA grading system. These graphs illustrate:

1- The normal group is very well defined in both grading system, but the normal group is not similarly presented in both systems. While the normal group is noticeably confined mainly to grade 1 and 2 (normal and mild changes respectively) in the HFA system; some normal subjects were recorded by the SNR grading system as having (G3-G4) which represented a poorer response from what we expected for a normal subject. This could be attributed to the marked variability in responses among normal population.

2- The glaucoma group was confined between grades 3 to 5 in the SNR system, while the HFA system showed wider range of glaucoma responses according to the grading system. Thus the visual field results can be classified into fine categories using the standard HFA, while the values of SNR does not offer this detailed range of responses.

3- The glaucoma suspect group showed very variable distribution among grades in the HFA grading system, while this group was clearly confined to two middle grades; grade 2 and grade 3, where the SNR values are lower than healthy but not very poor. Due to poor homogeneity of this group in terms of clinical findings suggesting a suspicious diagnosis of glaucoma, some patients were found to have repeated visual field defects while other findings were suspicious, which could have been considered as an early glaucomatous visual field defect rather than suspicious or unconfirmed glaucomatous loss.

## 4.10 THE MFVEP MULTICHANNEL RECORDING

This study used four channel recordings with a saddle-shaped electrode holder mounted on the occipital cortex. The four channels were in vertical, horizontal and diagonal orientations covering

the large area of visual cortex and inion landmark in the centre. It was found that the use of the hemifield sector analysis protocol in the multi-channel electrode montage was very helpful and yielded fewer errors in the waveform configuration. The new software (Roland Perimeter 6.1.4.11), which was created specifically for the purpose of conducting this study, was automatically detecting the best signal of the four channels based on the best SNR value recorded from each segment. Only the SNR values larger than 1.0 were included in the calculation. A single channel recording using this protocol would have made it more difficult to identify focal defects. The idea of comparing the superior and inferior hemifield sectors and hemi-rings is crucially dependent upon the presence of good waveforms representing true signals. Single channel recording would have been biased by the unique cortical convolution of subjects, and the orientation of the electrode in relation to area V1 in the visual cortex which would have made a big difference in the recorded waveforms in both superior and inferior hemifields. The problem of deciding what constitutes a local defect is not unique to the mfVEP. The HFA test has a similar problem. With the 24-2 HFA, it is common to highlight or define a cluster of significant points as abnormal if they collectively meet a set of criteria [(140)]. The main concern in mfVEP recording is to overcome the variability problem and the (normal) low amplitude variation in the periphery. Much of this concern could be resolved if a monocular test was carried out using a multi-channel recording system, and by carefully observing the outcome in order to rule out recording errors. Some authors used different approach to overcome the variability issues. Goldberg et al [(134)] suggested a cluster analysis approach similar to the HFA system. They labelled the mfVEP probability plot abnormal if three contiguous points exceeded 5% with at least one of the points exceeding 2%. This is also helpful, except that they used the amplitude as the measured parameter, which is by itself very variable and influenced by noise levels and occasional inaccurate identification of its value. However, in this study it was more practical to make an intrinsic reference to each location within the visual field which was found to be more effective than calculating probability figures for individual spots. Figure (28) shows one example of high noise where no clear signal could be identified from one study subject. The existence of too much noise in almost all segments should prompt to a connection error rather than true signal depression, and that this error should be checked and resolved by checking connection of wires and electrodes. Figure (29) shows two examples on how recording errors can sometimes be very confusing to the operator if not carefully picked up and managed. Many operators are already influenced by the knowledge that mfVEP recordings are variable across the whole field so they deal with errors lightly and believe it is either normal variation or counted it as false negative/positive results. Despite the efficiency of mfVEP tests to detect early glaucomatous visual field defects, it is very

important to provide good recording conditions and pay attention to the false positive errors. Figure (30) shows segments of almost flat waveforms or totally extinguished. In these segments, the SNR value is either below 1.0 or slightly above it. These focal depressions are most likely impeding a very small true signal, reflecting poor response. The underlying cause of this poor signal could be related to a glaucomatous defect or other reasons. It was not possible to identify the cause in this study, although if it was consistent and repeatable it would be considered as a glaucomatous defect if other findings can support it such as clinical assessment. These pure noise recordings were not included in the averaging process of the hemifield sector analysis in order not to bias the average SNR towards a low response by using the pure noise segment. It was beyond the limits of this protocol to compare each segment instead of sectors to their fellow corresponding ones in the opposite hemifield. Most of the time it was found that these small depressions were not found in the opposite hemifield, such as the example in figure (40), which can point to a possible focal glaucomatous visual field defect. It is important to emphasize that creating normative data for mfVEP for a group of individuals will not be of any use to other researchers working on different group of individuals, due to the high variability among subjects in their recording patterns, which is influenced by recording setups and individual anatomical variations. Good repeatability of any test is crucial if it is considered in monitoring and follow up of diseases, hence the importance of using good reference for normality in mfVEP, either this reference will be the fellow eye or, as in this study, the opposite hemifield. A repeatable mfVEP monocular test with multichannel electrode montage is currently considered the best approach for implementing mfVEP in the objective assessment of glaucomatous visual field defects.

## 4.11 NORMAL VARIATION OF MFVEP RESULTS

In this study some known normal variations were found and confirmed as such by the reversed polarity across the horizontal midline as shown in figure (31). As noted in many research studies [39,120,144,146,161], the responses from the upper and lower field are reversed in polarity in the majority of subjects. This reversal in polarity is consistent with the known anatomy of V1. The cortical magnification factor also was confirmed in the results as shown in figure (32), where SNR values in peripheral locations were lower than central ones. The location of the cortical region in relation to the recording electrodes is a crucial factor. That is why multichannel recording is considered in recent mfVEP testing protocols, to maximize the signal detection process at its highest representation. It has also been shown that the signals are smaller just below the

horizontal meridian than just above it [39,131]. Aine et al [167] provided one possible explanation. They recorded magnetically evoked potentials to focal stimuli in normal subjects. They were able to localize the various sources of the responses with dipole modelling. They found that occasionally if the field just below the horizontal meridian projects to the lower bank of the calcarine cortex, which is the case in many individuals, then the mfVEP signals from the region below the horizontal meridian, would be in the fold of the calcarine. This orientation will give the cells a more perpendicular location relative to the recording electrodes making the signal weaker and smaller just above the midline. Attention should be paid to mfVEP interpretation not to expect fixed foveal location in all mfVEP tests. Otherwise this finding could be easily interpreted as focal foveal depression when the classic waveform is missed at the expected location.

## 4.12 APPLICATION OF MFVEP IN CHILDREN

In this study no children were included in the study groups. In order to evaluate this new analysis protocol it was important to obtain the highest level of cooperation and lowest reliability errors. Enrolling children in this first assessment did not seem to provide this requirement, however a future assessment of this protocol should include different age groups including children. Overall, one of the benefits of using mfVEP visual field assessment is its feasibility and that it can be offered to children at an early age. The test does not require any active participation or cognitive skills from the subject to be performed, thus making it a good tool for visual field assessment for children who are able to take the test and cooperate effectively during the test procedure. There are different models of commercial mfVEP test equipment available that offer the test to children; such as the AccuMap® from Australia. Yukawa et al [187] performed mfVEP tests on many children to localize homonymous hemianopsia visual field defects. He concluded that the objective evaluation of visual field defects through multifocal visual evoked potentials might be useful in children in whom conventional perimetry is difficult. However, normative data about children and study of the VEP maturation responses at different age groups is essential if such a test would be implemented in children. Balachandran et al [120] studied the maturation of multifocal VEP in normal children in order to utilize their results clinically. They provided evidence that mfVEP shows an age related maturation in the visual pathway, which is characterized by unique timeframe of maturation and development for both amplitude and latency. They concluded that the mfVEP test could be performed by children as early as 5 years of age, which can provide important visual field information objectively.

## 4.13 THE GAP BETWEEN ANATOMICAL AND FUNCTIONAL GLAUCOMATOUS DAMAGE

Diagnosis of glaucoma has been always a clinical decision supported by groups of positive findings confirming visual field loss or presence of solid risk factors. Isolated risk factors do not form a good basis for confirming diagnosis unless definite and reproducible visual field loss is confirmed. Thus, diagnosis of glaucoma could be attributed to two main sources of evidence; the coexistence of specific risk factors known to be responsible or co-existence with glaucomatous optic neuropathy, and the presence of reproducible and reliable visual field defects. For many decades the assessment of the visual field was the main cornerstone of glaucoma diagnosis, where different tools and equipment were developed over the years to increase the accuracy of detection of these visual field losses, decrease the intra and inter-test variability, and improve test taking conditions that can produce highly reliable results. There has been always a link existing between test reliability and repeatability. When a visual field test is reliable, this simply means that the subject's subjective responses were accepted as a true reflection of their visual field. If the test is reliable on multiple occasions and yields a good level of accuracy and consistency then it is considered repeatable or reproducible. The issue of subjectivity versus objectivity in the assessment of visual field has passed through multiple steps of debate. Subjective visual field tests that directly related to the patient's responses and their level of cooperation were the major method of assessment over the years, and it is still considered the most important tool which has been developed and thoroughly investigated throughout the world. These subjective methods were simply telling the clinician how the patient sees his world in the patient's own words. However many sources of errors and pitfalls appeared through the evolution of these methods. Some were overcome by algorithmic modifications such as the SITA standard test, which was originally created to improve test-taking time and solve some reliability errors. Other errors, such as variability have remained attached to the body of these tests, despite repeated attempts to solve them or improve its figures. However, the huge normative database and records of physiological and pathological visual field results were accumulated over the years and formed a solid reference for these tests. That made standard SAP tests the gold standard test and this is something unlikely to change in the near future, as there is no available single test can give as much information about the visual field as the current SAP testing protocols. The introduction of objective testing for the assessment of visual field was a breakthrough in the understanding and management of glaucoma. Both structural and functional objective tests were extensively studied over the past two decades, and these tests showed very promising results on both levels of glaucoma diagnosis; the early

detection and the confirmation of existing field defects. It was well documented that anatomical losses precede functional visual field defects [1,2,11,203,204]. In addition, this fact is what made several forms of objective structural assessment of the retinal nerve fibre layer and optic nerve head a useful tool for glaucomatous visual field assessment. The relationship between the functional and structural changes due to glaucoma has been extensively debated, especially those related to the shape of and metric parameters of optic nerve head and the SAP indices [175,222-224]. In this work the correlation between structural and functional assessment of the visual field was not investigated, only two functional tests were assessed; the gold standard SAP and the mfVEP objective perimetry. Nevertheless, objective perimetry represents another sensitive and specific test that appears to be able to distinguish between normal and glaucomatous visual fields accurately, with higher sensitivity than the SAP standard test. This supports the idea of combining two tests in the assessment of visual field; the gold standard SAP and an objective perimetry test in the form of mfVEP.

## 4.14 MULTI-TEST COMBINATION STRATEGY

### 4.14.1 Combination of SAP and mfVEP

The use of SAP tests to confirm or rule out glaucomatous visual field defects has been the gold standard for visual field assessment for decades. The concept of subjective versus objective visual field assessment has been a source of debate in many studies. The term subjective: refers to the standard form of visual field testing using the Humphrey Field Analyser (HFA) or any other equipment that carries out SAP testing protocols. The subjectivity comes from the major dependency of the accuracy of the results on the patient's responses and answers to the test stimuli. Despite this, it has been well established that conventional visual field testing using SAP protocols are almost always influenced by intra and inter-test variability [11], yet it is still the reference test. Traditional and conventional methods for visual field testing are dependent on the subject's personal and intellectual factors; the level of cooperation, comprehension and accuracy of responses are all determinant factors that shape the reliability of SAP. For these reasons standard automated perimetry is somewhat difficult for both young children and elderly subjects [73]. One reported problem that influences the ability of SAP tests to determine progression of glaucomatous visual field defects is that the measurements become less repeatable as the disease advances and defects increase in depth or size [10,17,188-190]. In response to this shortcoming in progression monitoring, major improvements in the analysis software has led to more accurate

threshold detection and the introduction of new methods of perimetry algorithms that improved the progression analysis of SAP [191-195]. However, despite these improvements, there is a debate about the reliability of SAP in general, and with the introduction of mfVEP, the question extends to include whether mfVEP as an objective test could offer more reliability figures, and thus improve the repeatability of visual field assessment. SAP often gives rise to results which are categorized as suspicious or unconfirmed. The sector analysis protocol used in this research was very helpful in highlighting possible focal depression when it is not identified by the SAP test. However, it should be remembered that the mfVEP test has not been validated by a large scale clinical trial and as such, usually clinicians will not rely on these results alone when making a decision as to whether or not to start a treatment. The combined use of both tests; SAP and mfVEP in the category of suspicious and unreliable patients can add weight to the clinical status of the patient, especially when both agree on outcome. Figures 46-48 show examples of suspicious visual field tests that do not match with the clinical picture of the patient and still not strong enough to establish a diagnosis. The hemifield sector analysis was performed for these patients and it showed significant depression of multiple sectors corresponding with the SAP visual field test. These results when used in combination can support each other and point to a possibly true underlying glaucomatous visual field defect that need attention and modification of management plan.

### 4.14.2 Combination of mfERG and mfVEP

Since the early introduction of mfERG it has shown a very high correlation with many retinal diseases. Many studies tested the ability of mfERG technique to detect photoreceptors dysfunction, macular dystrophy, and acquired retinal diseases such as diabetic retinopathy and drug toxicity. The combination of both mfERG and mfVEP was studied in some research work, highlighting the benefit of retinal sensitivity in the diagnosis of glaucomatous visual field defects. Although this combination was suggested as a theoretically plausible idea to combine retinal sensitivity that covers 50-60$^{o}$ of visual field with the mfVEP responses of V1 visual cortex, the origin of the mfERG did not reflect any part of the visual system that could be influenced by glaucomatous visual field damage. The mfERG is a direct measure of retinal sensitivity, and the signal originates mainly from the outer retinal layers, mainly the photoreceptors dominated by the cones. There is not sufficient literature or research evidence that can suggest a direct effect of glaucoma on the photoreceptors. On the contrary, even for patients who have very advanced glaucomatous visual field defects with its classic periphero-central constriction of the visual field,

they still maintain good central vision, even if a small central island of vision is all that is preserved. Furthermore, the mfERG failed to prove any ganglion cell participation in the formation of its signal, whereas on the retinal level some glaucomatous changes might occur progressively. Hood et al [112] has investigated in depth the contribution of ganglion cell and inner retina in the mfERG by performing pharmacological dissection of the retinal layers, using Tetrodotoxin (TTX), which blocks the sodium, based action potentials. They found that ganglion cell contribution was detected in humans when contrast ratio of stimulus was set to 50% instead of the standard 100%, which could again raise the question about optimum testing protocols and settings used in mfERG to selectively acquire ganglion cell contribution. More recently, and in an attempt to include ganglion cell responses to the mfERG waveform configuration, multiple modification were added to the standard protocol of mfERG included adding bright white flash as an intermittent stimulus of different intensities and temporal resolution [108-110,115,116]. However, adding these white flashes to the mfERG protocol will not make the pattern stimulus itself, which is the standard method of testing, responsible for such contribution, it will be the simple flashes that could be obtained by other simpler methods responsible for this ganglion cell contribution. Adding mfERG to the gamut of glaucoma assessment seems to be unpractical idea for many reasons; most importantly is that it is another lengthy and inconvenient test similar to the mfVEP, and most likely adding the two tests will not be a very plausible idea to clinicians or to glaucoma patients.

### 4.14.3 Combined structural and objective visual field test

Several imaging techniques and technologies have become available for evaluating the structural changes in glaucoma by assessing the optic nerve head morphometric measurements or the retinal nerve fibre layer thickness. In addition, these structural modalities have shown good level of agreement with SAP tests with excellent reproducibility and reliability in well documented glaucomatous visual field defects [212-214,226,227]. It was shown by Nomoto et al [228] that other non-conventional methods of visual testing such as the FDT (frequency doubling technology), flicker perimetry (critical fusion frequency technique) and SWAP (short-wavelength automated perimetry) had higher sensitivity in early detection of glaucomatous visual field defects, in particular flicker perimetry. However, they found that the OCT nerve fibre layer analysis showed much greater sensitivity for early detection of glaucomatous changes. What makes the need for structural analysis to be added to the routine assessment tools of glaucoma is the high test-retest variability confirmed by many studies. This shortcoming of current perimetric techniques

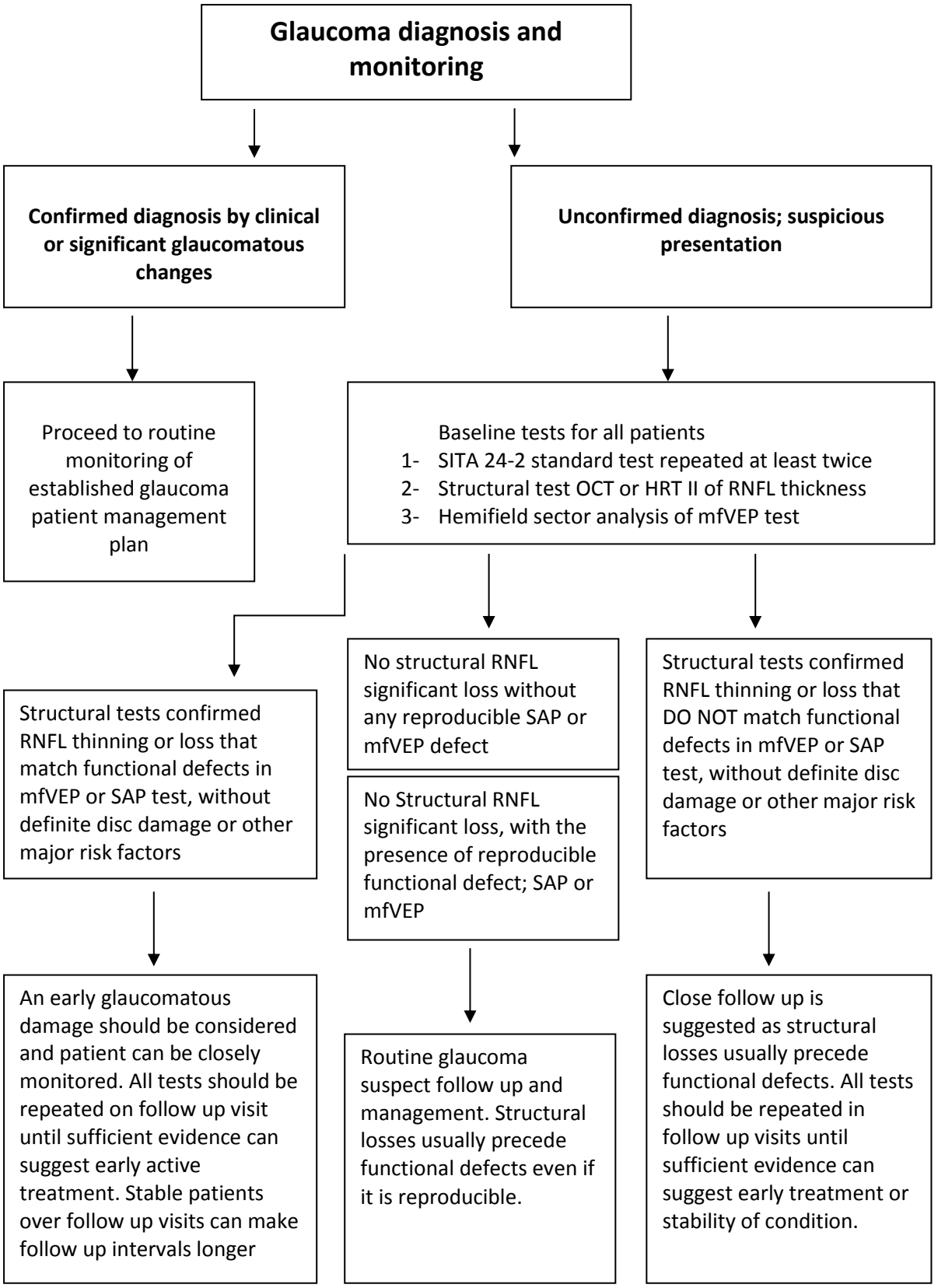
encouraged the development of other alternate methods such as mfVEP and structural analysis. The introduction of accurate anatomical assessment of glaucoma changes, especially the thickness of the retinal nerve fibre layer, was a breakthrough in the early detection of nerve damage. Clinically, it would be extremely helpful to have an agreement between a functional assessment of the visual field, such as HFA or mfVEP, and structural assessment of optic nerve head and retinal nerve fibre layer thickness. The combination between these tests can overcome the shortcoming of each individual test such as poor reliability or the SAP learning curve. Many studies [206-211] have shown significant correlation between optic nerve morphology and nerve fibre layer thickness and the standard visual field parameters. When a highly sensitive and specific test like the mfVEP used in combination with a structural test such as the scanning laser ophthalmoscope, it could provide very valuable information on the patient's visual field, confirming glaucomatous visual field defects at its early stage. Both structural and mfVEP tests can still be used as supporting tests to SAP, or in its absence when it is not reliable or undoable, and give reliable results on the visual field.

### 4.14.4 The best combination approach

Diagnosis of glaucoma and effective monitoring are considered the major concerns and objectives, in almost all of the research studies conducted in the field of glaucoma management. Visual field assessment is still the main monitoring tool that reflects progressive glaucomatous functional loss. Recently there are new methods of directly and indirectly assessing the effect of glaucoma on functional vision; most widely used are the objective structural and functional tests. There have been major advances in the structural assessment of optic nerve head and retinal nerve fibre layer thickness analysis over the past decade that made these tests more reliable, do its assessment against good normative data and credible normal range of measurements, and most importantly its high reproducibility and repeatability that make it one of the best reliable test can be used for monitoring of glaucomatous changes. The results of this research confirmed the reliability of the mfVEP in detecting glaucomatous visual field damage, and confirming a normal visual field, which is concordant with previous studies assessing the mfVEP for use in glaucoma evaluation. There is a growing new trend in the field of glaucoma diagnosis and management towards using different types of tests and combining different methods in glaucoma diagnosis and management. This new trend is not up to the level of standardization or setting new diagnosis and monitoring criteria until this moment, probably because there is still a need for more aggressive research to produce more evidence about the reliability and follow up validity of these tests. Many studies have evaluated

the best approach for the use of combined tests in glaucoma. Clinically, it would be extremely helpful to have an agreement between a functional assessment of the visual field, such as HFA or mfVEP, and structural assessment of optic nerve head and retinal nerve fibre layer thickness. Recently, Bouzkurt et al [212] evaluated the relationship between global indices of Humphrey SAP central 30-2 threshold test using SITA standard strategy, Humphrey Matrix 30-2 frequency doubling technology (FDT) threshold test, and HRT II parameters in ocular hypertensive (OHT) and glaucoma patients. The study group also tested other parameters and factors, like the level of agreement among the 3 groups specially in classifying study groups into normal and abnormal. They also evaluated the correlation between specific sector rim loss and its detection by other functional visual field assessment tests. They found that visual field global indices showed statistically significant correlations with most of the rim changes detected by HRT parameters. They suggested based on their findings that the use of either HRT or standard visual field assessment tests may show the first evidence of glaucomatous damage. They recommended the combined use of both tests to assess the optic nerve head analysis and visual field results in order to obtain better detection of early glaucomatous visual field defects. mfVEP was also tested in combination with HRT II in a study by Balachandran et al [213], they compared the data of functional and structural assessment where they found that both of them can detect existing glaucomatous visual field defects with limited correlation, ranged between 50%-77% depending upon the type of parameter used for comparison. Sensitivity and specificity of mfVEP were higher compared to HRT (93%, 96% - 79%, 92% respectively). They concluded that the mfVEP correlated better with the HFA than with HRT. However it remained important in their recommendations to use both functional and structural assessment for best monitoring of glaucomatous visual field defects. The mfVEP was also tested in combination with a different structural test; the optical coherence tomography measurements of the retinal nerve fibre layer thickness in a study done by Moschos et al [214], they tested two groups; normal controls and glaucoma patients who underwent the two tests. They found that the combined use of these two tools enhanced the ability to detect the glaucomatous visual field defects, and they suggested that it is useful to diagnose and monitor glaucoma using this combination. It was well documented that visual thresholds in patients with significant glaucoma based on SAP were found to be proportional to retinal ganglion cell density determined by histological cell counts [203-205]. In the early stages of glaucoma, where selective types of ganglion cells are more affected and first to be lost, SAP assessment is insensitive to this selective loss since the response of other types of ganglion cells mask a defect [203,204]. It has also been shown and proven that structural losses usually precede functional defects in glaucomatous damage [1,2]. This means that if a structural test shows some significant losses in an unconfirmed or

unreliable SAP test it is worth to take this seriously and closely monitor these patients until sufficient evidence is available. The high sensitivity of the mfVEP test can assist in these suspicious cases and alert clinicians to modify their management plan and make monitoring more often, and to add more functional and structural tests in the routine visits. The following strategy for combining different functional and structural tests in the diagnosis and management of glaucoma could be adopted:


Glaucoma diagnosis and monitoring
Confirmed diagnosis by clinical or significant glaucomatous changes
Unconfirmed diagnosis; suspicious presentation
Proceed to routine monitoring of established glaucoma patient management plan
Baseline tests for all patients
1- SITA 24-2 standard test repeated at least twice
2- Structural test OCT or HRT II of RNFL thickness
3- Hemifield sector analysis of mfVEP test
Structural tests confirmed RNFL thinning or loss that match functional defects in mfVEP or SAP test, without definite disc damage or other major risk factors
No structural RNFL significant loss without any reproducible SAP or mfVEP defect
No Structural RNFL significant loss, with the presence of reproducible functional defect; SAP or mfVEP
Structural tests confirmed RNFL thinning or loss that DO NOT match functional defects in mfVEP or SAP test, without definite disc damage or other major risk factors
An early glaucomatous damage should be considered and patient can be closely monitored. All tests should be repeated on follow up visit until sufficient evidence can suggest early active treatment. Stable patients over follow up visits can make follow up intervals longer
Routine glaucoma suspect follow up and management. Structural losses usually precede functional defects even if it is reproducible.
Close follow up is suggested as structural losses usually precede functional defects. All tests should be repeated in follow up visits until sufficient evidence can suggest early treatment or stability of condition.

## 4.15 CONCLUSIONS

1- The use of hemifield sector analysis protocol in mfVEP test has shown high sensitivity and specificity figure in detecting glaucomatous visual field defects (97% and 86% respectively), detecting glaucoma suspect patients (89% and 79% respectively), and identifying normal visual field accurately in 100% of all normal subjects. These figures confirm the test reliability in the use of glaucoma diagnosis and monitoring

2- This study did not evaluate the repeatability and inter-test variability as a good indicator for its future use in monitoring and follow up of glaucoma progression. However, many previous studies offered enough evidence that the mfVEP test can be used in monitoring of the progression of glaucomatous damage based on its high repeatability and low inter-test variability shown in these studies.

3- Future research work is needed on the hemifield sector analysis protocol to measure its effectiveness in detecting early glaucomatous visual field defects as suggested in this study by few examples, where the protocol showed possible early defects that need to be assessed longitudinally. This future research work should use the current sensitivity and specificity figures to design a cohort assessment of subjects with possible early glaucomatous defects.

4- The mfVEP results as an objective functional tool used in visual field assessment should be considered in the glaucoma management plan; especially in two situations: if the mfVEP results showed consistent and reproducible visual field defect, and if SAP test is unreliable due to any reason. The suggested role of mfVEP in glaucoma management plan should be limited in the time being, and based on the available research evidence, to adjustment of monitoring plan for glaucoma suspects and subjects with suspicious presentation without definite glaucomatous damage. Most certainly, future research work and availability of more evidence about the reliability of mfVEP test will enforce its role and expand its use as a crucial factor in designing management plan for glaucoma.

5- The monocular mfVEP test, using an intra-ocular reference values, can provide very valuable information about the visual field in glaucoma and glaucoma suspect patients. This type of data could be utilized in combination with SAP test and other structural objective assessment of the optic nerve head or retinal nerve fibre layer thickness to produce a comprehensive picture of the anatomical functional changes in glaucoma. This combination of tests is considered in many previous studies. This combination is possibly the best approach to monitor glaucoma patients and to follow up suspected patients with unconfirmed diagnosis.

6- The user friendly nature and ease-of-use of the hemifield sector analysis can encourage clinicians to consider the test done for their glaucoma and glaucoma suspect patients on an annual basis and as a baseline for new patients. Although some significant level of technical training and knowledge are needed to perform the test on patients, it is considered as any other high-tech test that requires initial application and technical training, but later on the use of the equipment is a straightforward process. Overall, the initial setup is costly and need some efforts but the data that can be obtained definitely worth the trial.

7- More research work still needed to make mfVEP more reliable and rectify its poor peripheral spatial resolution. If the test will be presented as a reliable tool for visual field assessment, especially in glaucoma patients, then the testing procedure should provide a high dynamic ratio stimulus, covers the entire visual field in a reasonably accepted spatial resolution that suits the nature of glaucomatous visual field defects, which is usually and by large start as small isolated islands of scotoma occupying 1-2 degrees of the visual field. This sort of coverage is not available at the moment in the mfVEP test, which discouraged many researchers to continue their work on its specifications, and others do not recommend it under these poor terms. The high sensitivity and specificity of the mfVEP are good enough to continue more research work, utilizing its advantages and important supporting role to the conventional visual field assessment methods available at the time being, and in the same time trying to improve its quality.

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# 6.0 APPENDIX 1

## Supporting Documents

**Paper 1**

# Paper 2

## THE ROLE OF HEMIFIELD SECTOR ANALYSIS IN MULTIFOCAL VISUAL EVOKED POTENTIAL OBJECTIVE PERIMETRY IN THE EARLY DETECTION OF GLAUCOMATOUS VISUAL FIELD DEFECTS

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# Paper 3

## EVALUATION OF HEMIFIELD SECTOR ANALYSIS PROTOCOL IN MULTIFOCAL VISUAL EVOKED POTENTIAL (MFVEP) OBJECTIVE PERIMETRY FOR THE DIAGNOSIS AND EARLY DETECTION OF GLAUCOMATOUS FIELD DEFECTS

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