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Comparative Study of the Use of Multifocal Electroretinogram versus Visual Field Testing in Evaluating Cases of Primary Open Angle Glaucoma

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ABSTRACT

Purpose: to compare between results of multifocal ERG and visual field in cases of primary open angle glaucoma.

Methods: 30 eyes with primary open angle glaucoma and 30 eyes of normal subjects were included in this study. Humpheray visual (SITA standard 24-2 strategy) and multifocal electroretinography were performed to all included patients and normal subjects.

Results: the patients group showed increased latency of the N and P wave of the multifocal ERG when compared to the normal subjects. No difference in amplitude of waves was found between the two studied groups. when comparing different stages of glaucoma (mild, moderate, and severe) according to the mean deviation of the visual field no difference was found in amplitude or latency of the waves produced by the multifocal ERG.

Conclusion: multifocal ERG was able to differentiate between patients with POAG and normal subjects in the form of prolonged latency of waves produced, but it was not able to differentiate between different grades of glaucoma. This makes it a good prognostic tool but not a diagnostic tool, where the automated visual field analyzer remains superior in diagnosing POAG.

Key words: multifocal ERG, Primary open angle glaucoma, visual field, lateny, amplitude, P wave, N wave.

INTRODUCTION

Primary open-angle glaucoma (POAG) is defined as a chronic slowly progressive optic neuropathy with characteristic forms of optic nerve damage and visual field loss. POAG lacks the identifiable contributing factors of the secondary open-angle glaucomas. Elevated intraocular pressure (lOP) is the most important risk factor for POAG. The elevation of intraocular pressure mainly is due to resistance of aqueous outflow through the trabecular meshwork (TM), which is the main drainage pathway of aqueous humor. The biological changes in the cells and the extracellular matrix (ECM) of that drainage pathway cause an increase in IOP and the pathogenesis of POAG[1].

Conventional diagnostic approaches of POAG depend on the following: 1. Elevated intraocular pressure, 2. Angle of the anterior chamber, 3. Signs of optic disc damage, 4. Signs of affected Visual field. Usually, the diagnostic findings are not so obvious. In these cases, the patient is called a glaucoma suspect. Such patients require repeated assessments of the optic nerve, the IOP and the visual field at regular intervals with the frequency of visits depending on the index of suspicion[2].

Visual field is used to assess the individual's functional vision. Perimetry helps clinicians to identify glaucomatous loss as well as to stage the disease according to the severity of field loss. The 24-2 program with a size III stimulus is considered the most commonly used testing pattern and target size with the Humphrey Field Analyzer[3].

Advantages of automated perimetry include providing more sensitive and reproducible results, giving quantitative information, very easy to be used, and it can detect early glaucomatous

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damage. SITA (Swedish Interactive Threshold Algorithm) standard 24-2 is a kind of static perimetry presenting static stimuli of white light at certain points of the patient's visual field. It was developed for the Humphrey perimeter and uses a complex mathematical process to measure threshold values for each point in a certain location depending on the stimuli presented to it and the response of the nearby locations[4].

In 1993,Hoddap *et al.* classified glaucoma patients according to the value of the mean deviation of the visual field, that was a trial to determine the severity of glaucomatous damage, where The criteria for mild glaucoma include: 1- mean deviation less than -6, 2-a cluster of ≥ 3 points on the pattern deviation plot in an expected location of the visual field depressed below the 5% level, at least one of which is depressed below the 1% level, 3-all points in the central 5 degrees must have a sensitivity of at least 15 degrees. The criteria for Moderate glaucoma include:1- mean deviation ranging from -6 to -12, 2- ≥ 25% but <50% of points on the pattern deviation plot depressed below the 5% level, and ≥15% but <25% of points depressed below the 1% level, 3- at least 1 point within the central 5° with sensitivity of <15 dB but no points in the central 5° with sensitivity of <0 dB, 4- only 1 hemifield containing a point with sensitivity <15 dB within 5° of fixation.The criteria for severe glaucoma include: 1- Mean deviation more than -12, 2- ≥ 50% but <75% of points on pattern deviation plot depressed below the 5% level and ≥25% but <50% of points depressed below the 1% level,3- any point within the central 5° with sensitivity <0 dB 4- both hemifields containing a point(s) with sensitivity <15 dB within 5° of fixation[5].

Multifocal Electroretinogram: Visual electrophysiology is a group of tests allowing non-invasive monitoring of the function of different processing stages along the visual pathway. They are mainly objective tests for the visual function. By using appropriate stimulation and recording techniques, electrophysiology provides an excellent tool for selective monitoring of the function of rods, cones, retinal bipolar cells and ganglion cells (especially after the development of multifocal techniques), It measures retinal pigment epithelial transport and the electrical activity of the visual pathway V1 and higher centers. All these techniques have been used recently with glaucoma. Introduction of the multifocal ERG (mfERG) is viewed as a major advance in electrophysiological technology. Depending on advanced signal analysis, it provides a major breakthrough by measuring the spatial resolution of the light-evoked electrical responses from the retina. The mfERG as a photopic stimulus is mainly generated by the retinal bipolar cells, which are stimulated by the cones.The pattern is arranged in hexagons. They are scaled small for the macula and increasing in size as we go to the periphery. This is done to compensate for receptor density decrease. Steady fixation is essential.Pupil dilation is mandatory for routine applications. Test time is approximately 10 minutes. Signals are picked up with corneal electrodes; many types can be used because of the relatively low demands on optical imaging. The analysis depends mainly on cross-correlation with the local stimulus sequence in relation to the global retinal response, allowing local responses to be measured. Those responses are available as a converging series of non-linear approximations[6].

Similar to the traditional full-field ERG, the mfERG reflects contributions from various retinal cell types. The overall shape of the first-order kernel of the mfERG is thought to represent the effect of bipolar cells combined with a smaller effect from photoreceptors. The onset (hyperpolarization) of the OFF-bipolar cell starts just before the depolarization of the ON-bipolar cell. So, the leading edge of the N1 is generated by the hyperpolarization of the OFF-bipolar cell with small contribution from the photoreceptors. The shape of N1 is then altered by the onset of the ON-bipolar response. The recovery of the OFF-bipolar response occurs slightly after the peak of N1.Consequently, the leading edge of the P1 contains both the recovery of the OFF-response with the depolarization of the ON-bipolar cells. The peak of P1 occurs at the time when the recovery of the OFF-response has reached its positive peak and the contribution of the ON-bipolar has also reached its peak. The recovery of the ON-response mainly forms the trailing edge of P1.The magnitude of the mfERG response decreases with eccentricity and the reduction rate is slightly less in the nasal retina because of the higher cone density. As with many other assessments of visual function, ageing is characterized by significantly reduction of the mfERG responses[7].

Methodology:

This Study was conducted between April 2014 and January 2016. All participants were chosen from patients attending the ophthalmological outpatient's clinics of Ain Shams University. It included 15 Egyptian patients with bilateral POAG, their ages ranged from 35 and 50 years. Fifteen age and sex matched healthy subjects were included as a control group. The aim of our study is to evaluate the retinal function in patients with POAG of different stages using the multifocal electroretinogram and the visual field then, correlating the results of both tests.Visual field testing was performed using (Humphery Field Analyzer) for white-on-white (achromatic) condition. MfERG stimulation was performed with the Roland Consult RETI port/scan 21 (Roland Consult, Brandenburg, Germany). Responses were recorded monocularly using Hk-loop thread electrode, which was positioned in the inferior fornix of the conjunctiva. The pupil was dilated with 1 % tropicamide and the non-examined eye was occluded. Gold-cup reference and surface electrodes were applied to the subject's temple and forehead, respectively, using a conductive paste. Impedance < 10 kOhm. The visual stimulus array was driven on a CRT monitor consisting of 61-scaled hexagons.The size of the hexagons was scaled with eccentricity to elicit approximately equal amplitude responses at all locations. Each hexagon was temporally modulated between black and white according to pseudorandom binary m-Sequence with luminance of 100 cd/m^2 in white hexagons and 2 cd/m^2 in black hexagons. Normal room lighting was used. Subjects were asked to maintain fixation on the red fixation target at the center of stimulus matrix and refrain from blinking. Each session of recording took approximately four minutes to complete, a break was given after each 30 seconds of recording. Data from two full mfERG recording sessions were obtained for each subject and were automatically averaged.For each waveform, the amplitude and implicit time of the first negative trough (N1) and the first positive peak (P1) of the first-order kernel were determined. P1 amplitude was measured from the trough of the first negative wave to the peak of the positive wave while the implicit time was measured from stimulus onset to the first prominent response peak.

Two grouping configurations were used, rings and quadrants. The five rings groupings were five wave form grouping responses from five concentric rings. Ring (1) is the most central hexagons with radius of about 0.5 mm (1.7)°. Rings 2, 3, 4, 5, were responses of increasingly eccentric annuli of stimulus. The four quadrants grouping was four-wave form grouping response from superonasal, superotemporal, inferotemporal, inferonasal. Using averaging programs, all wave form amplitudes were scaled in nv/degree^2 (density – scaled average: (degree^2) reflects the angular size of the stimulus hexagons that produced the response).

Statistical analysis:

The results were statistically analysed using (Statistica software, version 8). Quantitative variables were expressed as mean ± SD, whereas qualitative variables were presented as numbers and percentage. Student's *t*-test was used to assess the statistical significance of differences between the two groups. Correlation analysis was performed by calculating Pearson correlation coefficient (r). Regression analysis was done to assess the different factors that influence MF-ERG five- rings grouping waveforms and four-quadrant grouping waveforms (Amplitude/area and MF-ERG five- rings grouping waveforms (Amplitude) and (latency). P Values were considered statistically significant if < 0.05.

RESULTS

Age of the control group ranged from 37 to 63 years with a mean of 50.57 years, while age of case group ranged from 37 to 71 years with a mean of 52.77 years. Regarding Age and Gender, No statistical significant differences were observed between the two groups. In the control group the best corrected visual acuity ranged from 6/6 to 6/18, while in the case group it ranged from 6/6 to 6/36. Highly statistical significant differences were observed between the two studied groups regarding VA & BCVA (p = <0.0001). IOP in the group 2 ranged from 32 to 10 mmHg with a mean of 18.93, while in group 1 it ranged from 11 to 18 mmHg with a mean of 13.63. IOP showed highly statistical significant differences between the two studied groups (p = <0.0001). Cup/Disc ratio in the glaucoma group ranged from 0.4 to 0.9, while in the control group it ranged from 0.2 to 0.5. The Cup/Disc ratio showed highly statistical significant differences between the two studied groups (p = <0.0001).The 2 groups were compared according to the Mean deviation of the visual field measured in decibels at the time of examination. Group one showed a mean -2.95db while group 2 showed a mean of -17.27db. The mean deviation (MD) showed highly statistical significant differences between the two studied groups (p = <0.0001) as shown in table (1).

The case group was subdivided into 3 groups according to the severity of glaucoma into Mild (group 2A), Moderate (group 2B) and severe (group 2C). 6 eyes had mild glaucoma, 8 eyes had moderate glaucoma and 16 eyes had severe glaucoma

Multifocal ERG results:

Group 1 (controls) and group 2 (cases) were compared to each other according to the multifocal ERG results, where the results included the amplitude and latency of the negative wave (N) and the positive wave (P) of the first order kernel of the mfERG, also the average Amplitude of P wave/ area (nV/deg^2) was measured .Results of the 61 location tested in the retina were grouped once in 4 quadrants (Q), and once in 5 rings (R).

Multifocal electroretinographic four-quadrant grouping response densities (nV/degree^2) showed no statistical significant differences between the two studied groups, and four-quadrant grouping amplitude (µV) of P1 showed no statistical significant differences between the two studied groups.

Multifocal electroretinographic four-quadrant grouping latency (ms) of P1 showed statistical significant differences between the two studied groups (p = <0.0001) in the 4 quadrants. Latency was longer in the glaucoma group as shown in table (2) and Fig.(1).

Multifocal electroretinographic four-quadrant grouping amplitude (µV) of N1 showed no statistical significant differences between the two studied groups while latency (ms) of N1 showed statistical significant differences between the two studied groups (p =0.01, 0.001 & 0.005 respectively) in the 3 quadrants (quadrants2, 3 & 4)as shown in table(3) and Fig.(2)

Multifocal electroretinographic of five-ring grouping waveforms (Amplitude/area) (nV/degree^2) showed no statistical significant differences between the two studied groups and, five-ring grouping waveforms of P1 amplitude (µV) of P1 showed no statistical significant differences between them. However, multifocal electroretinographic five-ring grouping waveforms latency of P1 latency (ms) showed statistical significant differences between the two studied groups (p = <0.0001, <0.0001& <0.0001 respectively) in the 3 outer rings (rings 3, 4 & 5).

Results of the visual field and mf-ERG in sub-groups of glaucoma:

The mean deviation (MD) in decibels(db) revealed a statistical significant difference between the three glaucoma groups, the mild(2A), moderate(2B) and severe(2C). Multifocal electroretinographic four-quadrant grouping response densities (nV/degree2) showed no statistical significant differences between the three glaucomatous sub-groups except for quadrant 1 & 3 as there is statistical significant difference between mild glaucoma group and moderate glaucoma sub-group (p = 0.02 & 0.02 respectively). The severe glaucoma sub-group showed no statistically significant difference when compared to other sub-groups. Multifocal electroretinographic four-quadrant grouping amplitude (μV) of P1 showed no statistical significant differences between the three glaucomatous groups except for quadrant 1 & 3 as there is statistical significant difference between mild glaucoma group and moderate glaucoma group (p = 0.02 & 0.02 respectively).

Multifocal electroretinographic four-quadrant grouping latency (ms) of P1 showed no statistical significant differences between the three glaucomatous groups in the 4 quadrants while, four-quadrant grouping amplitude (ms) of N1 showed statistical significant differences between the three glaucomatous groups in the quadrants 1, 2 &3 except that of quadrant 4. The four-quadrant grouping latency (ms) of N1 showed no statistical significant differences between the three glaucomatous groups in the 4 quadrants. Multifocal electroretinographic of five-ring grouping waveforms (Amplitude/area) (nV/degree2) showed no statistical significant differences between the three glaucomatous groups except for R3 & R5 as there is statistical significant difference between mild glaucoma group and moderate glaucoma group (p = 0.02 & 0.03 respectively) ,while the five-ring grouping waveforms of P1 amplitude (μV) showed no statistical significant differences between the three glaucomatous groups except for R3 & R5 as there is statistical significant difference between mild glaucoma group and moderate glaucoma group (p = 0.02 & 0.03 respectively). The five-ring grouping waveforms latency of P1 (ms) showed no statistical significant differences between the three glaucomatous groups.

Discussion:

The results of the mf-ERG four-quadrant response densities (nV/degree2)and amplitude of P1 showed no significant statistical differences between the two studied groups, while the latency of P1 was statistically significant longer in the glaucoma group compared to the control group (p = <0.0001). The four-quadrant amplitude of N1 showed no differences between the two studied groups, while its latency was longer in the glaucoma group compared to the control group (p = <0.0001).The five-ring grouping waveforms density (nV/degree^2) and amplitude of P1 showed no statistical differences between the two studied groups. The latency of P1(ms) was statistically longer in the glaucoma group in comparison with the control group (p = <0.0001, <0.0001& <0.0001 respectively) in the 3 outer rings (rings 3, 4 & 5).The four-quadrant grouping response densities (nV/degree^2) and amplitude (μV) of P1 showed no statistical significant differences between the three glaucomatous subgroups except for quadrant 1 & 3 as there was difference between the mild glaucoma group and the moderate glaucoma group (p = 0.02 & 0.02 respectively). The latency of P1 of the four quadrants showed no differences between the three glaucomatous subgroups. The four-quadrant grouping amplitude (ms) of N1 showed statistical differences between the three glaucomatous subgroups in the quadrants 1, 2 &3 except that of quadrant 4 (p = 0.02, <0.001, 0.03 and 0.86 respectively), However, it was not diagnostic as the amplitudes of moderate and severe groups were more than those of mild glaucoma group. Four-quadrant grouping latency of N1 showed no statistical differences between the three glaucomatous subgroups in the 4 quadrants. When comparing the results of the visual field and the multifocal ERG, the visual field was able to diagnose glaucomatous damage in the form of the presence of scotomas in the visual field, also it was able to determine the stage of glaucoma whether mild, moderate or severe according to the mean deviation of the results in decibels. Multifocal ERG showed increased latency of the P and N waves in the glaucoma patients, However it was unable to differentiate stages of glaucoma according to severity. **Chan and Brown**[8] conducted a study to compare the results of multifocal ERG in 18 eyes with POAG and 15 normal subjects. The results showed prolonged latency in the glaucoma group in the four quadrants and in the five rings display. The prolongation in latency decreases the eccentrically we go from the macula. They also found reduction in amplitude of waves in the glaucoma group compared to the normal group. The results of the present study match their results as regards the increased latencies in the glaucoma group. However we didn't find a statistically significant difference regarding the amplitude of the waves. **Hasegawa *et al.*** [9]study was conducted on 26 eyes of 14 patients with POAG and 26 eyes of 26 control subjects.The glaucoma group was classified into mild and severe glaucoma according to the severity of the visual field defects. Their results showed longer peak latencies of P1 and N1 waves in the POAG group in comparison to their control group. In agreement with our study, they found no statistically significant difference between both groups regarding the amplitude of waves. When comparing results according to severity of glaucoma, they found that the severe glaucoma group showed longer mean latency of both P1 and N1 waves than the group of mild glaucoma, They also found no statistically significant difference of the amplitude of waves in the glaucoma patients and the control. **Parisi *et al* .**[10] enrolled 24 POAG patients and 14 age-matched controls in their study. They found a statistically significant increase in the latency of P1 of the glaucoma group when compared to normal subjects, and a significant decrease in the amplitude density (nV/deg^2) in the glaucoma group. These results were statistically related to the Mean deviation of the visual field. **Rao *et al* .**[11] conducted a study on 97 eyes diagnosed with primary open angle glaucoma and normal tension glaucoma that were evaluated by Humphrey visual field, and mfERG. The global and averaged quadrant peak-to-trough amplitudes and latencies of the first trough (N1), the first peak (P1) of the mfERG were compared with visual field results in early,moderate and advanced glaucoma.The results showed that the amplitude of P1 and N1 waves were reduced in the early and moderate stages of glaucoma, while the latency of the P1 wave showed statistically significant reduction in severe stages of glaucoma. These results matched our study in the prolongation of latency of waves in the glaucoma group. However, there was no reduction of amplitude of waves in glaucoma patients. **Golemez *et al*.** [12] study included 126 eyes of 126 patients, and 30 healthy eyes (Group1), 28 eyes were diagnosed as glaucoma suspect (Group 2), 48 eyes as early glaucoma (Group 3), and 20 advanced glaucoma cases (Group4). Humphrey visual field analysis and mfERG were examined. They found statistically significant prolonged latency of N1 and P1 waves between advanced glaucoma patients and control group, and between advanced glaucoma patients and glaucoma suspects. They found decreased amplitude of the N wave in all grades of the glaucoma group in comparison with the normal group. These results matched our results in the prolongation of the latency of waves in glaucoma patients, but didn't match our results in the decrease of amplitude of N wave in the glaucoma group. In the present study, alterations of Latency of P1 and N1 waves in the four quadrants were able to indicate the presence of, but not differentiate the stages of glaucoma.

Conclusion:

Visual field testing showed the ability to diagnose and determine different stages of POAG, where the mean deviation can be used to classify glaucoma patients into mild, moderate and severe stages. Multifocal ERG revealed prolonged latency of the waves in the cases of POAG, but could not differentiate between different stages of glaucoma.

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Table (1): Clinical and demographic data of patients with healthy volunteers (group 1) and Glaucoma patients (Group2):

Parameter	**Group 1**	**Group 2**	**t**	**P**
	Mean ± SD	**Mean ± SD**		
Age (years)	50.57 ± 0.48	52.77 ± 0.67	-1.05	0.30
VA (LogMAR)	0.16 ± 0.06	0.55 ± 0.40	-4.86	<0.0001
BCVA (LogMAR)	0.06 ± 0.03	0.40 ± 0.31	-5.7	<0.0001
Refraction (sphere-Diopters)	0.53 ± 0.04	0.008 ± 0.04	1.27	0.21
Refraction (cylinder-Diopters)	-0.12 ± 0.05	-0.73 ± 0.05	1.56	0.12
Spherical Equivalent (Diopters)	0.46 ± 0.08	-0.34 ± 0.03	1.61	0.112
IOP (mm Hg)	13.63 ± 0.25	18.93 ± 0.22	-5.92	<0.0001
C/D ratio	0.29 ± 0.07	0.67 ± 0.19	-10.62	<0.0001
Mean deviation (dB)	-2.59 ± 0.33	-17.27 ± 0.05	7.22	<0.0001

LogMAR = logarithm of the minimum angle of resolution
IOP = Intraocular pressure
C/D ratio = cup/disc ratio
DB = decibels

Table (2): mf-ERG four-quadrant grouping waveforms latency of P1 in the two studied groups.

latency (ms)	**Group 1**	**Group 2**	**t**	**P**
	Mean ± SD	**Mean ± SD**		
Q 1	45.56 ± 1.26	46.87 ± 1.64	-3.47	<0.0001
Q 2	45.24 ± 1.27	46.55 ± 1.30	-3.94	<0.0001
Q 3	44.66 ± 1.21	46.14 ± 1.60	-4.07	<0.0001
Q 4	44.68 ± 1.98	46.55 ± 1.86	-3.76	<0.0001

Table (3): mf-ERG four-quadrant grouping waveforms latency of N1 in the two studied groups.

latency (ms)	**Group 1**	**Group 2**	**t**	**P**
	Mean ± SD	**Mean ± SD**		
Q 1	25.59 ± 1.64	26.21 ± 1.77	-1.41	0.16
Q 2	25.24 ± 1.92	26.52 ± 1.82	-2.6	0.01
Q 3	24.10 ± 1.74	25.74 ± 1.93	-3.45	0.001
Q 4	24.79 ± 2.28	26.44 ± 2.07	-2.93	0.005

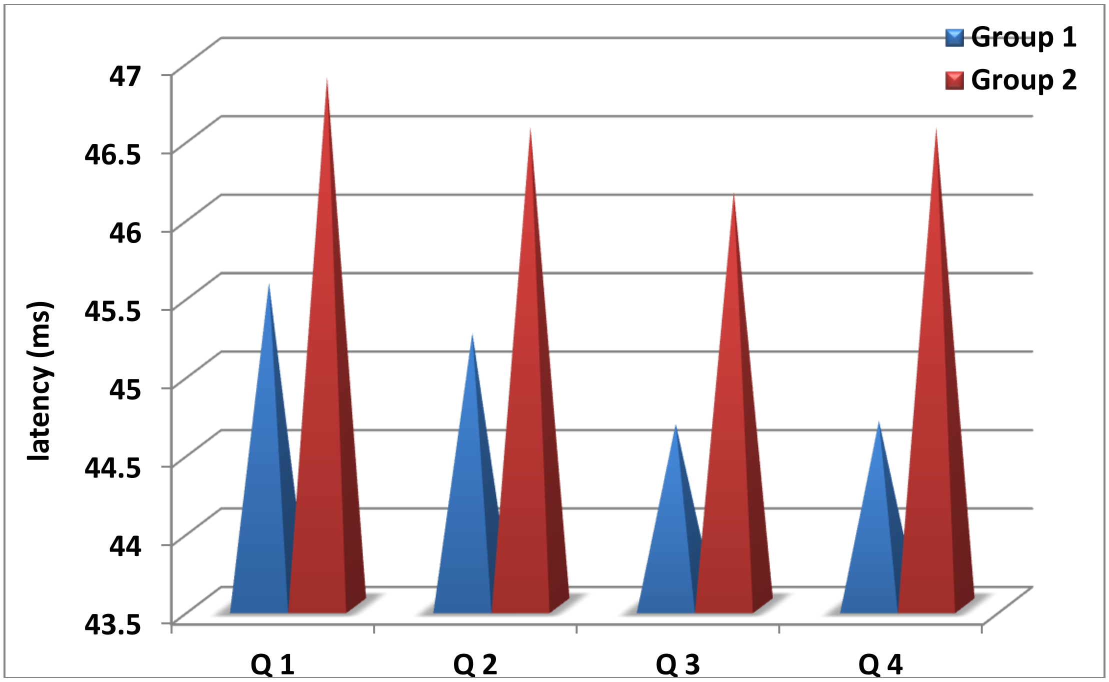

Figure (1): mf-ERG four-quadrant grouping waveforms latency of P1 in the two studied groups.

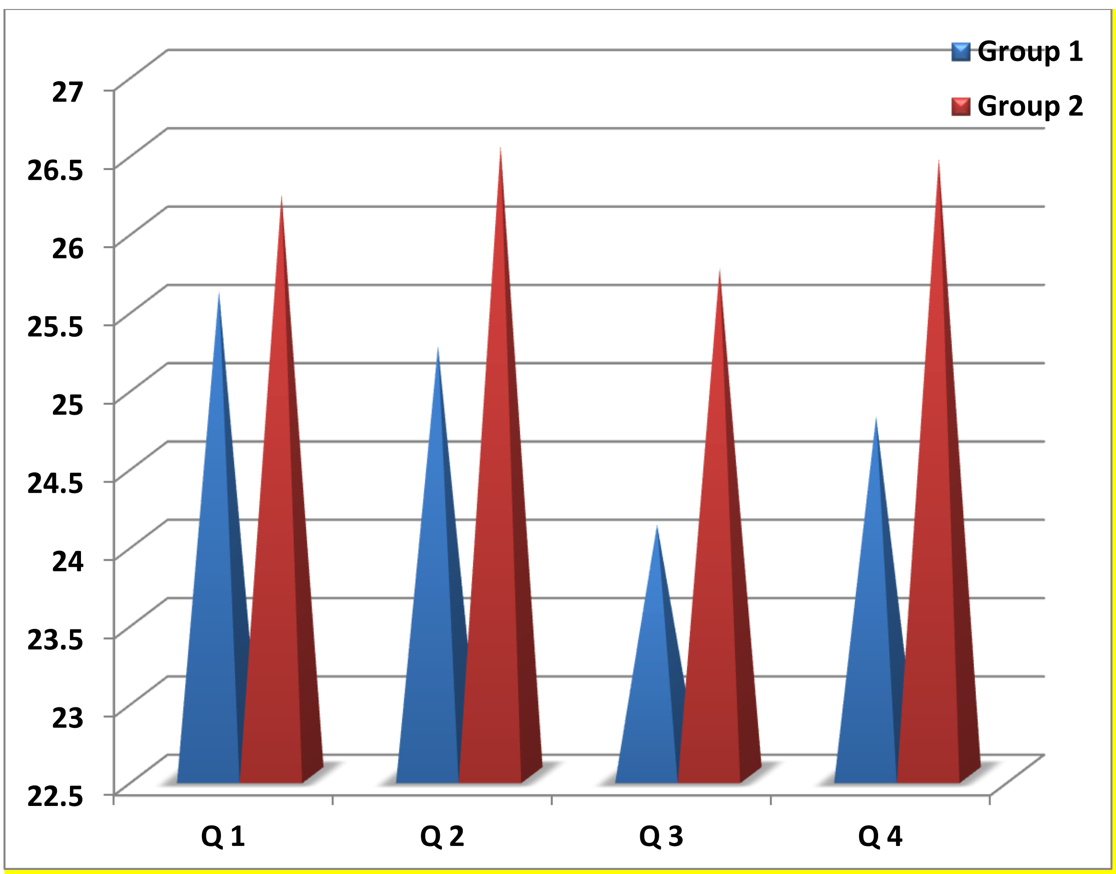

Figure (2): mf-ERG four-quadrant grouping waveforms latency of N1 in the two studied groups