Original Article

Principal components' analysis of multifocal electroretinogram in retinitis pigmentosa

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Aims: To determine waveforms of multifocal electroretinogram (mfERG) in patients with retinitis pigmentosa (RP) contributing significantly to the overall retinal response by using principal components' analysis. **Settings and Design:** Prospective, non-randomized, single-visit, observational, case-control study from a single tertiary ophthalmic center. **Materials and Methods:** Patients with various forms of RP underwent mfERG testing for a period of one year. The first-order kernel responses of RP cases were compared with concurrently recruited healthy controls. **Statistical Analysis Used:** Parametric data was analyzed using the unpaired t test for differences between the implicit time and amplitudes of cases and controls. Principal components' analysis was done for each implicit time and amplitude in cases with RP using the Varimax rotation method. **Results:** From March 2006 to March 2007, 24 cases with typical RP (56%, 47 eyes) were included in the final analysis. Their mean age was 33.7 years (19-69±15.5 years). Comparison of latencies and amplitudes among RP cases with log MAR acuity ≤ 0.18 and those > 0.18, revealed significant difference in the implicit time (P1) in Ring 2 only (*P*=0.028). Two components (predominently from Ring 1 and 2) each contributing 66.8% and 88.8% of the total variance in the data for latencies and amplitudes respectively, were seen. **Conclusions**: The first two rings of the mfERG contributed to the variance of waveforms in RP, irrespective of the visual acuity and poor visual field results.

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Retinitis pigmentosa (RP) is a generic name for a group of hereditary disorders characterized by night-blindness, impaired dark adaptation, and a progressive visual field loss, that often leads to blindness.[1,2] In an extensive epidemiologic study conducted in South India, the prevalence was greater than other reports from the Western populations and that of the conservative estimate of 1 in 4000.[2] In another study done in India, a genetic and segregation analysis of RP[3] patients showed that 9% of cases were autosomal dominant, 36% were autosomal recessive, 3% were x-linked recessive, 44% were isolated instances, and 8% cases were of undetermined genetic type. The full-field electroretinogram (ERG) in RP typically shows a marked reduction of both rod and cone signals, although rod loss generally predominates; a and b waves are reduced since the primary site of disease is at the photoreceptors or the retinal pigment epithelium (RPE). The ERG is usually abnormal in infancy or early childhood, except for some of the very mild and regional forms of RP.

Recently, the multifocal electroretinogram (mfERG) has proven to be a valuable diagnostic aid.[1,4,5] The mfERG technique developed by Sutter *et al*., permits a localized measurement and mapping of the retinal response,[4] thus providing an objective assessment of the central retinal function. The typical waveform of the primary mfERG response (also called the first-order response or first-order kernel *K*1) is a biphasic wave with an initial negative deflection followed by a positive peak. There may be a second negative deflection after the peak. The preferred designation is to label these three peaks N1, P1 and N2 respectively. There is some homology between this waveform and the conventional ERG, but they are probably not identical.

Thus the designations 'a wave' and 'b wave' are not recommended. The N1 response amplitude is measured from the starting baseline to the base of the N1 trough; the P1 response amplitude is measured from the N1 trough to the P1 peak. The peak implicit times of N1 and P1 are measured from the stimulus onset [Fig. 1]. However, due to the presence of these complex array of waveforms [Fig. 2], there exists no clear opinion on which of these waveforms should be especially sought by the clinician to assess retinal function in advanced cases of RP.[5] The objective of this study was to analyze the patterns of mfERG in RP patients and identify the definitive waveforms responsible for the majority of retinal function in these patients. Identification of such waveforms would isolate areas of preserved retinal sensitivity even in advanced cases of RP.

Materials and Methods

This was a prospective, non-randomized, single-visit, observational case-control study of consecutive cases with RP and their age-matched controls. Twenty-four consecutive patients (47 eyes) with RP from a single tertiary referral ophthalmic center in Mumbai city from March 2006 to March

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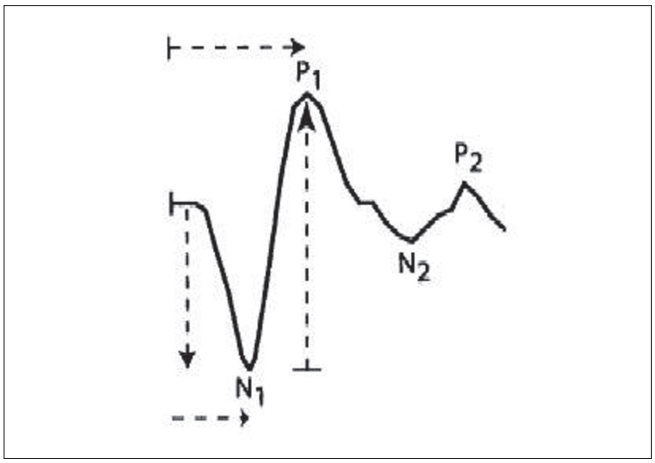


Figure 1: Diagram of an mfERG response to show designation of the major waveforms, and the recommended method for measuring amplitude and implicit time (time-to-peak)

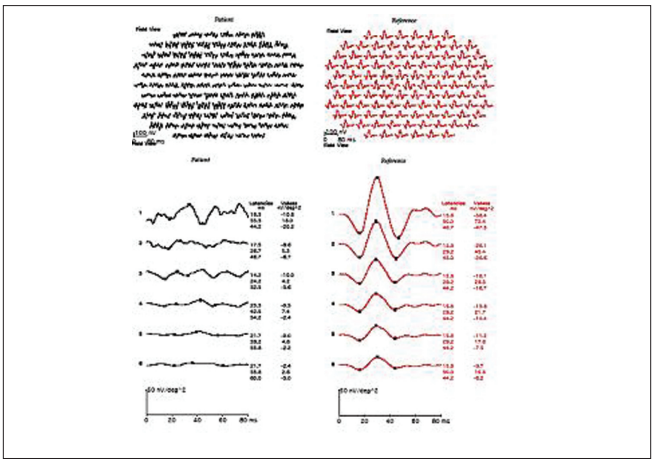


Figure 2: Trace arrays and responses from each ring from a patient of RP

2007 were studied. In the same period, healthy volunteers willing for mfERG testing and without any ocular disease affecting test outcome were recruited as controls (13 subjects, 26 eyes).

Inclusion criteria were patients with non-syndromic (without any systemic association) or syndromic (with systemic association, e.g. deafness in Usher's syndrome) RP who could complete the mfERG testing fully and exclusion criteria were atypical RP (e.g. Sector RP, Pericentral RP and Inverse RP), significant media opacities; cystoid macula edema (visualized by ophthalmoscopy and/or optical coherence tomography); or the presence of a maculopathy, glaucoma, nystagmus, myopia greater than -6.00 diopter sphere (DS) or any systemic disease that could affect vision or the capacity to perform the tests.

Best-corrected distance visual acuity (BCVA) was assessed on a log MAR (logarithm of minimum angle of resolution) scale, using the back-lit Early Treatment Diabetic Retinopathy Study (ETDRS) charts. Slit-lamp examination and intraocular pressure measurement (applanation) were performed in all patients before testing. Stimulation and primary analysis were performed using a stimulus camera-refractor unit (VERIS, ver. 5.1; Electrodiagnostic Imaging [EDI] San Mateo, CA). The pupils were fully dilated with tropicamide 1% eye drops and phenylephrine 5% eye drops, instilled twice over 15 min. Bipolar Burian-Allen contact lens electrode with a built-in infrared illuminator (Hansen Ophthalmic Development Laboratory, Coralville, IA, and EDI) was placed on the corneal surface of the eye according to instructions in the full-field ERG or Pattern ERG standards of the International Society for Clinical Electrophysiology and Vision (ISCEV).[6,7] Subjects were placed comfortably in front of the screen or 21" Ultra-High Luminance Stimulator. Fixation was monitored by direct observation. In patients requiring refractive correction, lenses were typically placed in a holder positioned in front of the eye. Because lenses alter the relative magnification of the stimulus, the viewing distance was adjusted to compensate, in accordance with the guidelines provided by the manufacturer. Subjects were adapted in ordinary room light for 15 min before testing. Prior exposure to bright sun or fundus photography was avoided. Longer adaptation was needed after such exposure. The stimulus array was within central 30 degrees on either side of fixation which consisted of 103 elements. Total time for recording was typically about 3 min 38 sec and was divided into eight segments of 27.29 sec each so that subjects could rest between runs if necessary and also for discarding any poor record (from noise, movement or other artifacts) without losing prior data. Full-field ERG testing was tried in all patients. Standards laid down by the ISCEV were followed during testing.[6,7]

Humphrey visual field test was done where possible with a large target as the visual fields were severely restricted in the majority of the patients (only seven patients could complete the test).

The study methods adhered to the tenets of the declaration of Helsinki and the International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines were followed during the period of the study.

Parametric data was analyzed using the unpaired t test for differences between the latencies and amplitudes of cases and controls. Principal components' analysis was done for each latency and amplitude in cases with RP using the Varimax rotation method. Cronbach's alpha was >0.7 and only those eigenvectors with values more than 1 were computed in the final analysis. Variables were excluded from analysis at communality values >0.4 in more than one component. The SPSS Version 13 software (SPSS Inc., Chicago, IL, USA) was used for analysis. Statistical significance was assumed at $P<0.05$.

Results

From March 2006 to March 2007, 43 patients of RP were seen in this tertiary ophthalmic referral center in Mumbai city. Out of these, 24 (56%) were included in the final analysis. A predominant number of the cases were male (22, OR 13.68, 95% CI 3.85 to 49.88 $P<0.01$). The mean age of the patients was 33.7 (range 19--69 SD $\pm$15.5 years). Isolated RP (Simplex) was seen in 20 patients (83.3%), while autosomal recessive inheritance was seen in three patients (12.5%), and autosomal dominant inheritance was seen in one case (4.2%).

The mean log MAR visual acuity of the RP patients was

1.37 (range 0 to 3 ± 1.29). The latencies of the waveforms (N1, P1, N2) in the first three rings of the first-order kernel were compared with similar waveforms of controls (independent t test). Comparison of amplitudes of cases and controls in the first-order kernel were similarly done [Table 1]. Comparison of latencies and amplitudes among RP cases with log MAR acuity ≤ 0.18 and those with > 0.18, revealed a difference in the implicit time (P1) in Ring 2 only (P=0.028). Results are shown in Table 2. As the differences in the mean latencies and amplitudes of the first-order kernel of cases with RP and controls were significant in most part of the waveforms [Table 1], principal components' analysis (PCA) was done to demonstrate which waveforms in the first-order kernel were responsible for these differences.

Latencies in all three rings of all waveforms (N1, P1, N2) when analyzed in the correlation matrix were found to have an adequate mean sampling score (Kaiser Mayer Olkin, Measure of sampling adequacy) KMO, MSA score =0.653, MSA>0.5), also, the Bartlett test for sphericity was significant (P<0.01). Eigenvalues were computed for each component. The first two components in the latencies contributed to 66.82% of the total variance of the data [Fig. 3]. A similar covariance matrix computed for the amplitudes revealed MSA score of 0.611 with a significant Bartlett's test P<0.01. Eigenvalues of the first two components were more than 1 and these components contributed to 88.8% of the total variance in the data [Fig. 4].

The PCA showed that the first and second rings in the first-order kernel contributed to most of the variance in the data.

Table 1: Comparisons of latencies and amplitudes of cases with retinitis pigmentosa and controls

Variable	Controls N(%) 25 eyes	RP cases N(%) 47 eyes ±	*P* value
Ring 1			
N1 Latency	13.83	12.99	0.759
N1 Amplitude	-25.08	-5.75	0.001*
P1 Latency	30.07	30.5	0.053
P1 Amplitude	29.10	3.30	<0.001*
N2 Latency	56.77	63.68	0.110
N2 Amplitude	-21.43	-1.80	0.001*
Ring 2			
N1 Latency	14.08	17.38	0.132
N1 Amplitude	-10.19	-3.25	0.001*
P1 Latency	27.70	44.12	<0.001*
P1 Amplitude	14.97	2.86	<0.001*
N2 Latency	48.99	65.17	<0.001*
N2 Amplitude	-9.27	-0.87	<0.001*
Ring 3			
N1 Latency	14.12	20.90	0.011*
N1 Amplitude	-6.03	0.60	<0.001*
P1 Latency	27.36	44.62	<0.001*
P1 Amplitude	9.90	1.80	<0.001*
N2 Latency	45.66	68.03	<0.001*
N2 Amplitude	-6.21	-1.58	<0.001*

± = One patient was unable to perform the test in one eye, • = Significance at P <0.05

Humphrey Field Analysis (HFA) was possible in seven cases of which five revealed tubular vision. Flash ERG was unrecordable in all cases except one with predominant cone response.

Discussion

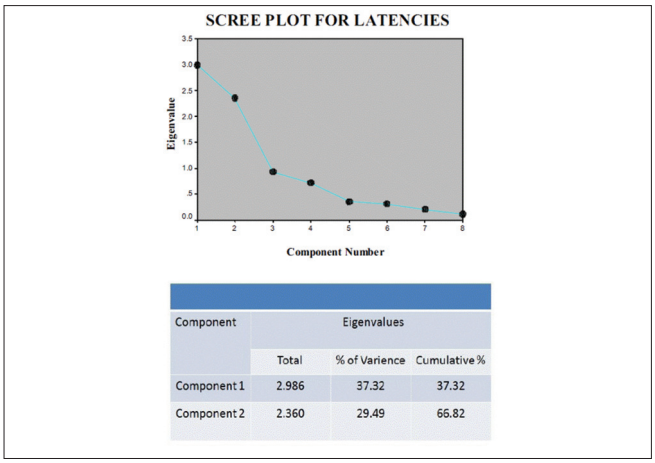
Our study showed male preponderance and also poor mean visual acuity. The comparison of the current study with a similar study of cases and controls, done by Seeliger *et al*,[8] shows notable differences in the cases as well as controls when considering latencies and amplitudes [Table 3]. Controls in this study showed lower latency and lower amplitude responses in all rings of the first-order kernel compared to their Western counterparts, despite having nearly similar selection criteria as regards age, refractive error and visual acuity [Table 3]. The exact cause of this difference is unknown.

Birch *et al.*,[9] in their study on the yearly rate of visual loss in patients with RP, observed that the rod-mediated ERG amplitudes declined faster compared to cone-rod dystrophy patients. Also, this rate of progression was seen to be different among the various inheritance patterns of RP patients. The current study being a single-visit observational series, we were unable to prognosticate the loss of mfERG waveforms in our patients or predict poor visual outcome in them over time.

Chan *et al.*,[10] in a study of mfERG response densities in patients with RP, noted a general depression in both the macular (Ring 1) and pericentral retina (Ring 3) with little increase in response with stimulus luminance increase. They

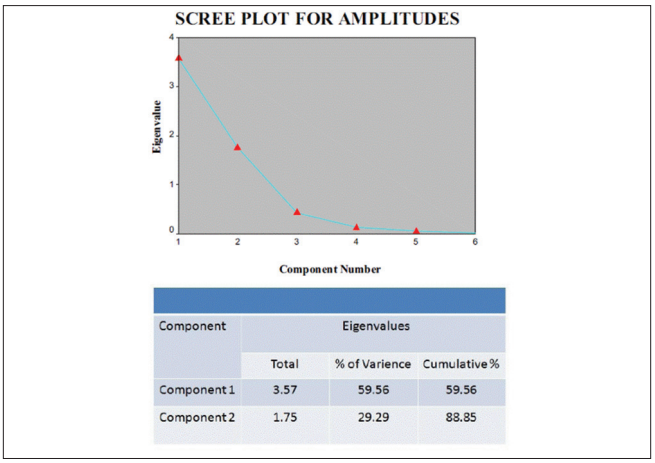
Table 2: Comparisons of latencies and amplitudes of cases with retinitis pigmentosa

Variable	Visual acuity 6/9 better $_{16}$N(%)	Visual acuity Worse than 6/9 $_{31}$N(%)	*P* value
Ring 1			
N1 Latency	13.17	12.90	0.935
N1 Amplitude	-11.24	-2.92	0.300
P1 Latency	32.08	41.82	0.102
P1 Amplitude	1.21	4.38	0.750
N2 Latency	59.11	66.04	0.163
N2 Amplitude	4.00	-4.80	0.299
Ring 2			
N1 Latency	13.50	19.38	0.126
N1 Amplitude	-4.96	-2.37	0.372
P1 Latency	34.73	48.96	0.028*
P1 Amplitude	2.48	3.08	0.894
N2 Latency	61.19	67.22	0.201
N2 Amplitude	1.03	-1.85	0.411
Ring 3			
N1 Latency	20.00	21.36	0.793
N1 Amplitude	-0.668	-0.56	0.945
P1 Latency	39.69	47.17	0.287
P1 Amplitude	1.38	2.06	0.769
N2 Latency	64.69	69.76	0.194
N2 Amplitude	-0.31	-2.23	0.270



Component	Eigenvalues		
	Total	% of Varience	Cumulative %
Component 1	2.986	37.32	37.32
Component 2	2.360	29.49	66.82

Figure 3: Scree plot for latencies, Component 1=P1 and N2 of Ring 1 and complete Ring 2 waveform (N1, P1, N2) Component 2= Ring 3 (N1, P1, N2)



Component	Eigenvalues		
	Total	% of Varience	Cumulative %
Component 1	3.57	59.56	59.56
Component 2	1.75	29.29	88.85

Figure 4: Scree plot for amplitudes, Component 1 = N1 wave amplitudes of Ring 1 and 2, Component 2 = N2 wave amplitudes of Ring 1 and Ring 2

Table 3: Comparisons of implicit times and amplitudes of Cases and Controls

	Ring 1		Ring 2		Ring 3	
	Lat	Amp	Lat	Amp	Lat	Amp
Seeliger						
Cases	31.7	28.9	30.0	7.9	32.5	3.1
Controls	31.7	58.15	30.0	26.9	30.0	16.3
P values	0.067	<0.001	0.84	<0.001	0.0038	<0.001
Present study						
Cases	38.3	13.0	40.8	6.1	40.8	2.3
Controls	26.7	30.8	27.5	18.0	27.5	12.6
P values	0.053	<0.001	<0.001	<0.001	<0.001	<0.001

noted that in patients with relatively preserved vision, some photopic responses were derived from the macular area. We concur with these findings and also note that despite extremely poor vision in some RP cases, we did elicit recordable mfERG responses.

Interestingly, Seeliger *et al.*,[8] in their assessment of the diagnostic potential of mfERG in cases with RP note that eccentricity-dependent changes in amplitude and latencies were indicative of the affected and non-affected areas. They observed a loss of mfERG response density (amplitude/area) in patients with RP that was significant in all five eccentricity groups while the implicit time was significantly elevated from the third eccentricity group onwards. In comparison with this study, the present study shows that there are notable differences in the magnitude of correlation observed. We noted a statistically significant difference in all amplitudes across the retina (all rings) and significant difference in all latencies as well except the central retina (Ring 1) which was not statistically significant [Table 3].

PCA was used as a data averaging technique.[11] It was preferred over multiple regression analysis in this study due to the large number of wave forms with binary values to every wave {amplitude(nv) and latency(ms)}. Also, because the variables in the waveforms (Amplitude and Latencies) are not cell-specific, it would have been meaningless to do mathematical averages.

With PCA, the arithmetical value of cellular responses is maintained in its original state and no assumption of cellular origin is made. Comfrey *et al.*,[11] recommend a minimum sample size of 300 for best results from PCA. Several investigators debate the optimum sample size for this analysis, with recommendations of 50 to 400 being reported.[12,13] Others however insist on maintaining a subject to item ratio of 5:1 for best use rather than sample size.[13] We adhered to the latter principle during the analysis.

Isolation of orthogonal (unrelated) vectors from the given data was possible because the mfERG response is not a true cellular response but a computer-averaged waveform.[14,15] Nevertheless, our endeavor was to identify important waveforms that contribute maximally (at least 60%) to the composite retinal response. Two components were found contributing significantly to the response densities and latencies. Ring 1 and Ring 2 responses were found contributing maximally to the overall response. Such cellular preservation in the macular and para-macular areas is an important contributor to the central retinal responses (Rings 1, 2, 3).

The duration of RP and the mode of inheritance was varied in our group of patients; this could be thought as a drawback.[14,15] However, considering the overwhelming number of simplex (sporadic) cases and the slow rate of visual field loss in RP cases, we reckon our study was reasonably uniform on those two counts. The appropriateness of PCA in a small sample size is debatable,[13] however, as discussed earlier, we maintained a good case to variable ratio in the final analysis.

In summary, this study highlights that mfERG responses of the central and para-central retina contribute maximally to the overall retinal response irrespective of the visual acuity in RP.

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