



### Normal Values of Standard Full Field Electroretinography in Healthy Subjects in Northern Iran

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| ARTICLE INFO  | ABSTRACT   |
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| <p><b>Article type:</b><br/><i>Original Article</i></p> <p><b>Bullet points:</b></p> <ul style="list-style-type: none"> <li>• <i>The variability of ERG normal values in different populations</i></li> <li>• <i>Localization of ERG normal values in the north of Iran</i></li> </ul> <p><b>Article history:</b><br/>Received: 15 Jan 2016<br/>Accepted: 16 Apr 2017<br/>Available online: 8 Aug 2017<br/>CJNS 2017; 3 (10): 135-142</p> | <p><b>Background:</b> Electroretinography (ERG) is known as a diagnostic test for evaluating the performance of different layers of the retina. Its normal values may be different in different populations.</p> <p><b>Objectives:</b> Determining the normal values of ERG parameters in patients presenting to an ophthalmologic center in Guilan in northern Iran.</p> <p><b>Materials and Methods:</b> Fifty-five people presenting to a teaching hospital affiliated to Guilan University of Medical Sciences were studied in autumn and winter 2013. The subjects were diagnosed as healthy in the eye examinations and were examined in terms of the values of amplitude and latency time of ERG waves in accordance with international guidelines for clinical electrophysiology of vision.</p> <p><b>Results:</b> In this study, 55 patients with a mean age of 35±14 years were studied (40% males with a mean age of 36.00±15.4 years; 60% females, with a mean age of 34.5±14 years). The following results were obtained: amplitude of scotopic 0.01=149±144 μV, scotopic 3=317±157μV, photopic=129±86 μV, 30-Hz flicker=134±38 μV, OS2=25.6±13.9 μV, Latency time scotopic 0.01=53.5±29 sec, Latency Time Scotopic 3=34.3±15.2 sec, N1=13.59±2 sec, P1=18.63±1 sec, N2=21.3±1 sec, P2=26.1±2.7 ms, N3=31.1±4 sec, P3=33.8±4.7 sec, N4=40.9±6.4 sec, P4=44.3±6.4 sec, Latency Time photopic=22.9±7.7 ms, and flicker=54.3±7.5 ms. Men and women were not significantly different in terms of the variables. Left and right eyes, as well as age groups were not significantly different (<math>p=0.05</math>).</p> <p><b>Conclusion:</b> The results of the current study differed from other studies in terms of the values of amplitude and latency times. Therefore, it is necessary to standardize ERG for each center.</p> <p><b>Keywords:</b> Electroretinography, Reference Values</p> |
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## Introduction

**E**lectroretinography (ERG) is a useful method for evaluating performance of various cells in the retina (1-3). ERG evaluates photoreceptor cells along with the health of other cells, including cells in the retinal pigment epithelium and Müller cells, which are essential to create proper minor ERG responses (4). Responses obtained from standard ERG constitute five categories: [1] responses from rod cells, [2] responses from cone cells, [3] combined responses from rod and cone cells, [4] oscillatory potentials, [5] responses to a flicker stimulus (5-8). ERG results vary depending on the way it is conducted, so an international standard was developed in 1989 by the International Society for Clinical Electrophysiology of Vision (ISCEV) that is revised every few years and its standards are used by various centers (1,2). Accordingly, many attempts have been made to standardize ERG responses in different centers around the world, which is very difficult due to the influence of several factors, including age, sex, and population under study, refractive error, test environment, the type of device and the operator (9-14). Parvaresh *et al.* (15) extracted the normal values of standard ERG in an Iranian population, and the data was analyzed in terms of age, sex, amplitude, latency time and other characteristics. As many patients require ERG and that normal range of data was not available in the North of Iran, it appeared necessary to conduct a separate standard ERG study on the population in that region, using the ERG device in an academic ophthalmic hospital in the north of Iran affiliated to Guilan university of medical sciences, and examine the impact of a variety of factors including genetic factors in order to have a proper

interpretation of the obtained results and to facilitate the treatment.

## Materials and Methods

Among the eligible volunteers (10 to 60 years old) presenting to our hospital for refractive errors in the fall and winter of 2013, 55 people were selected through non-probability convenience sampling after thorough examination including the best corrected visual acuity (20/20 or better) by Snellen E chart at 4 meters, examination of the anterior segment with slit lamp (Slit Lamp, Haag Streit, BQ900, Switzerland), IOP measurements with applanation tonometry device (Haag Streit, Bern, Switzerland), and retinal examination with a 90D Non-Contact Slit Lamp Lens and indirect ophthalmoscope (Welch Allyn, USA), color vision using Ishihara cards and pupil reflexes (Direct, Consensual, Relative afferent pupillary defect).

Inclusion criteria were refractive errors (hyperopia, myopia, astigmatism) less than  $\pm 3$  diopter, normal vision with best correction (20/20 or better), normal color vision, normal anterior segment, normal eye pressure (12 to 20 mmHg), normal retinal examination, and absence of nystagmus. All the subjects were living in Guilan Province. Exclusion criteria were sensitivity to mydriatic drops, not cooperating enough in wearing lenses, abnormal color vision, diagnosis of retinal abnormalities, degenerative diseases of the retina such as night-blindness, achromatopsia, Leber, retinal inflammation, cone-rod dystrophies, nystagmus, a long history of using drugs that disrupt the retinal function (4), any known systemic and eye diseases and abnormal pupil size. Ophthalmic examinations were performed by a

specialist of retinal disorders. All measurements were performed by a trained optometrist, expert in electroretinography, and all patients underwent the five-step ISCEV standard test [5-8]. ERG was recorded for each eye separately. The ISCEV ERG Ganzfeld Program that is a part of the device (Roland Consult, Electrophysiologic Diagnostic Systems, Wiesbaden, Germany) was used to record ERG. The device's software was RETI port science 32. The device was calibrated before testing. After briefing the participants about the test and obtaining their informed consent, their pupils were dilated by dripping tropicamide 1% in their eyes twice with a 5-minute interval. Then 20 minutes after the last drop and ensuring the dilation of the pupil, the subjects were seated in front of the ERG device and the recording electrodes were installed including ground electrode on the forehead, and reference electrodes on the right and the left temporal area. Then their eyes were blindfolded and they were seated blindfolded in a dark room for 20 minutes. Then the blind folds were removed and active electrodes were placed on their cornea in the form of contact lenses (Jet Electrode). Impedance was controlled to ensure correct placement of the electrodes. After placing the participants' head in the right position, ERG responses were measured in the dark adapted state according to the ISCEV standard including:

- 1) Rod cell responses (Dark-adapted 0.01) was the first measured signal after adaptation to the dark. Its stimulus was a dim white flash with a power of  $0.01 \text{ cd}\cdot\text{sm}^{-2}$  with a minimum interval of 2 seconds between the flashes.
- 2) Combined response of rod-cone cells triggered by a flash with a power of  $3 \text{ cd}\cdot\text{s}\cdot\text{m}^{-2}$  with a minimum interval of 10 seconds between the flashes.
- 3) Oscillatory potentials:

They might be observed on the ascending limb of the b-wave or the flat part of the wave in the form of electrical oscillations. However, they cannot be drawn for all patients. Oscillatory potentials components are characterized as N1 and P1, N2 and P2, N3 and P3, N4 and P4 and OS2 on the printed ERG report. After recording the responses in the dark adapted state, the lights were turned on and the subjects were seated in the room for 10 minutes. Then the subjects' head was correctly placed and ERG responses were recorded in the light adapted state under ISCEV standard including:

- 1) Cone cells responses to a flash with the power of  $3 \text{ cd}\cdot\text{sm}^{-2}$  with a minimum interval of 0.5 seconds between the flashes.
- 2) Responses to a flashing stimulus with about 30 stimulations per second (30 Hz). After recording the response and the analysis of the results, each result was printed. Data analysis was also conducted separately on each eye. The subjects were divided into 5 age groups. Sample size is estimated for main index by a pilot study ( $n=10$ ). Data obtained from the patients was summarized in tables and analyzed using statistical methods in SPSS version 21. Statistical methods included mean, 95% confidence interval, standard deviation, and median. The Kolmogorov-Smirnov test was used to determine the normality of variables in order to compare the waves, amplitude and latency time in terms of demographic variables of sex, age group, and eye. The Independent t-test was used to compare the variables that followed a normal distribution, and the Mann-Whitney U test was used for variables that did not follow a normal distribution. One-way ANOVA was used to compare the variables that followed a normal distribution based on the age groups, and the Kruskal-Wallis H test was used for

variables that did not follow a normal distribution. Furthermore; 1) all examinations and measurements were performed with the consent of the participants. 2) No charges were incurred on the participants in the study. 3) Any complications caused by placing the electrodes including corneal abrasion were treated until full recovery free of charge.

## Results

In this study, 55 patients with a mean age of  $35 \pm 14$  were evaluated, 40% were male (N=22) with a mean age of  $36 \pm 15.4$  and 60% were female (N=33) with a mean age of  $34.5 \pm 14$ . In assessing the ERG components, median and standard deviation of amplitudes

and latency times were calculated (tables 1 to 4). The present study also compared ERG components based on sex, age group, and the eye. In general, the comparative study of variables in terms of age groups showed no significant difference in any of the studied variables. The comparison of variables in terms of the eye showed no significant difference. The comparison of variables between male and female subjects showed no significant difference except in P1L (0.01), P1R (0.024), P3R (0.035), N3R (0.025), P4R (0.022) and N4R (0.035), ( $p < 0.05$ ). Tables 1 to 4 show the values obtained in ERG components based on the variables in this study.

**Table 1.** The median and 95% confidence interval of amplitude ( $\mu\text{V}$ ) of ERG waves according to age groups in women

| Age (years)   | 10-20   |         | 21-30   |           | 31-40   |         | 41-50   |         | 51-60   |         |
|---------------|---------|---------|---------|-----------|---------|---------|---------|---------|---------|---------|
|               | Right   | Left    | Right   | Left      | Right   | Left    | Right   | Left    | Right   | Left    |
| Rod a-wave    | 9       | 8       | 11      | 7         | 6       | 10      | 8       | 8       | 8       | 9       |
|               | 4-12    | 5-15    | 6-13    | 4-13      | 4-20    | 223-316 | 224-294 | 222-303 | 239-304 | 245-296 |
| Rod b-wave    | 212     | 179     | 180     | 190       | 187     | 166     | 169     | 163     | 191     | 193     |
|               | 103-232 | 152-225 | 139-221 | 149-268   | 146-232 | 145-209 | 139-219 | 123-197 | 168-230 | 161-223 |
| Max a-wave    | 178     | 279     | 256     | 258       | 249     | 243     | 261     | 274     | 278     | 271     |
|               | 192-308 | 225-331 | 213-354 | 202-3-353 | 240-312 | 223-316 | 224-294 | 222-303 | 239-304 | 245-296 |
| Max b-wave    | 379     | 399     | 439     | 402       | 425     | 414     | 506     | 503     | 461     | 464     |
|               | 308-521 | 312-527 | 375-594 | 336-571   | 398-507 | 359-490 | 426-557 | 407-556 | 423-477 | 419-486 |
| os2           | 21      | 26      | 20      | 20        | 23      | 24      | 19      | 26      | 21      | 28      |
|               | 13-38   | 13-38   | 9-43    | 9-43      | 19-29   | 19-29   | 18-33   | 18-33   | 15-51   | 15-51   |
| Cone a-wave   | 47      | 46      | 44      | 51        | 47      | 45      | 47      | 51      | 52      | 54      |
|               | 38-59   | 40-65   | 39-59   | 37-59     | 41-58   | 40-54   | 37-61   | 44-59   | 42-58   | 34-94   |
| Cone b-wave   | 193     | 197     | 190     | 209       | 204     | 209     | 209     | 237     | 213     | 226     |
|               | 161-258 | 175-258 | 159-247 | 169-262   | 136-225 | 147-220 | 135-257 | 164-267 | 167-224 | 198-237 |
| 30 Hz Flicker | 134     | 147     | 141     | 129       | 124     | 101     | 163     | 148     | 151     | 144     |
|               | 116-197 | 103-182 | 115-187 | 112-178   | 105-139 | 95-150  | 93-173  | 80-161  | 100-161 | 93-153  |

**Table 2.** The median and 95% confidence interval of latency time (ms) of ERG waves according to age groups in women

| Age (years) | 10-20 |       | 21-30 |       | 31-40 |       | 41-50 |       | 51-60 |       |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Eye         | Right | Left  | Right | Left  | Right | Left  | Right | Left  | Right | Left  |
| Rod a-wave  | 84    | 76    | 81    | 84    | 79    | 78    | 80    | 83    | 79    | 80    |
|             | 72-85 | 72-81 | 76-87 | 76-88 | 75-91 | 76-86 | 73-82 | 76-86 | 74-82 | 76-81 |
| Rod b-wave  | 19    | 24    | 26    | 23    | 24    | 26    | 30    | 25    | 26    | 25    |
|             | 21-69 | 20-29 | 20-32 | 20-27 | 23-28 | 24-30 | 22-32 | 21-28 | 21-27 | 19-27 |
| Max a-wave  | 22    | 23    | 22    | 22    | 22    | 22    | 21    | 21    | 21    | 21    |
|             | 15-31 | 16-30 | 19-22 | 20-22 | 19-22 | 18-22 | 18-22 | 18-21 | 19-21 | 19-21 |
| Max b-wave  | 51    | 52    | 48    | 48    | 47    | 44    | 45    | 46    | 46    | 44    |
|             | 43-68 | 43-70 | 45-49 | 41-48 | 41-47 | 40-47 | 44-47 | 44-47 | 42-51 | 41-50 |
| OP N2       | 21    | 21    | 21    | 21    | 21    | 21    | 21    | 21    | 21    | 21    |
|             | 21-21 | 20-22 | 19-22 | 20-22 | 20-21 | 19-21 | 20-21 | 20-21 | 20-21 | 20-21 |
| OP P2       | 25    | 25    | 25    | 25    | 26    | 26    | 25    | 25    | 25    | 25    |
|             | 24-25 | 24-26 | 23-30 | 23-31 | 25-26 | 24-26 | 24-26 | 24-25 | 24-26 | 24-26 |
| Cone a-wave | 21    | 21    | 22    | 21    | 22    | 21    | 21    | 21    | 21    | 21    |
|             | 14-15 | 14-15 | 14-16 | 14-16 | 14-15 | 15-15 | 14-16 | 14-15 | 15-16 | 14-15 |
| Cone b-wave | 31    | 31    | 47    | 47    | 47    | 47    | 46    | 47    | 47    | 44    |
|             | 30-31 | 29-31 | 29-31 | 29-31 | 30-31 | 29-30 | 29-31 | 29-30 | 29-31 | 29-30 |
| 30 Hz N1    | 61    | 61    | 61    | 61    | 61    | 61    | 61    | 61    | 61    | 61    |
|             | 60-61 | 60-62 | 60-61 | 60-61 | 60-61 | 60-61 | 60-62 | 60-63 | 50-63 | 51-62 |
| 30 Hz P1    | 25    | 25    | 25    | 25    | 26    | 26    | 25    | 25    | 25    | 25    |
|             | 46-50 | 45-50 | 44-50 | 45-49 | 45-49 | 46-50 | 46-51 | 46-52 | 36-49 | 37-51 |

**Table 3.** The median and 95% confidence interval of amplitude ( $\mu$ V) of ERG waves according to age groups in men

| Age (years) | 10-20   |         | 21-30   |         | 31-40   |         | 41-50   |         | 51-60   |         |
|-------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Eye         | Right   | Left    | Right   | Left    | Right   | Left    | Right   | Left    | Right   | Left    |
| a-wave      | 28      | 18      | 13      | 7       | 12      | 16      | 11      | 8       | 9       | 6       |
| Rod         | 5-12    | 6-15    | 7-17    | 0.2-14  | 7-17    | 9-23    | 10-39   | 4-13    | 1-18    | 5-13    |
| b-wave      | 229     | 231     | 321     | 119     | 148     | 242     | 192     | 208     | 117     | 182     |
| Rod         | 189-274 | 34-328  | 50-356  | 131-313 | 180-376 | 80-364  | 28-312  | 29-370  | 132-238 | 95-300  |
| a-wave      | 332     | 367     | 355     | 351     | 236     | 259     | 263     | 272     | 321     | 333     |
| max         | 319-334 | 281-315 | 83-547  | 106-531 | 44-417  | 46-417  | 118-439 | 51-518  | 269-373 | 283-383 |
| b-wave      | 531     | 544     | 634     | 535     | 369     | 400     | 406     | 435     | 419     | 455     |
| max         | 131-931 | 226-861 | 83-930  | 96-995  | 176-827 | 146-946 | 306-546 | 274-605 | 355-517 | 347-582 |
| OS2         | 29      | 29      | 34      | 34      | 26      | 26      | 26      | 26      | 23      | 23      |
|             | 97-156  | 97-156  | 1-59    | 1-59    | 3-55    | 3-55    | 1-59    | 1-59    | 2-59    | 2-59    |
| a-wave      | 66      | 68      | 57      | 49      | 34      | 42      | 61      | 60      | 54      | 53      |
| cone        | 46-86   | 36-174  | 29-84   | 4-119   | 7-61    | 15-99   | 17-94   | 39-82   | 47-62   | 48-57   |
| b-wave      | 236     | 239     | 241     | 252     | 162     | 184     | 235     | 237     | 179     | 187     |
| cone        | 96-375  | 80-398  | 105-328 | 89-383  | 50-273  | 37-331  | 61-310  | 155-319 | 121-237 | 94-280  |
| 30 Hz       | 132     | 132     | 170     | 132     | 93      | 75      | 124     | 155     | 127     | 123     |
| Flicker     | 115-195 | 105-185 | 135-195 | 21-226  | 63-123  | 47-197  | 72-195  | 40-251  | 74-164  | 75-193  |

**Table 4.** The median and 95% confidence interval of latency time (ms) of ERG waves according to age groups in men

| Age (years) | 10-20  |        | 21-30  |       | 31-40  |       | 41-50 |        | 51-60 |       |
|-------------|--------|--------|--------|-------|--------|-------|-------|--------|-------|-------|
| Eye         | Right  | Left   | Right  | Left  | Right  | Left  | Right | Left   | Right | Left  |
| a-wave      | 81     | 83     | 78     | 80    | 85     | 64    | 80    | 80     | 81    | 78    |
| Rod         | 1-164  | 26-87  | 56-101 | 64-97 | 63-107 | 48-81 | 70-91 | 59-102 | 64-94 | 62-95 |
| b-wave      | 30     | 24     | 28     | 24    | 23     | 18    | 25    | 28     | 23    | 25    |
| Rod         | 26-87  | 5-43   | 0.2-45 | 8-31  | 0-45   | 6-42  | 13-38 | 10-45  | 8-38  | 10-38 |
| a-wave      | 20     | 21     | 22     | 22    | 21     | 19    | 21    | 21     | 19    | 20    |
| max         | 0.4-38 | 0.4-39 | 14-27  | 14-27 | 12-24  | 13-25 | 13-25 | 13-25  | 15-21 | 12-27 |
| b-wave      | 47     | 47     | 38     | 38    | 43     | 42    | 47    | 47     | 47    | 47    |
| max         | 17-81  | 29-67  | 36-39  | 24-56 | 40-43  | 25-57 | 42-50 | 38-50  | 34-56 | 38-53 |
| OP N2       | 21     | 21     | 21     | 21    | 21     | 21    | 21    | 21     | 21    | 21    |
|             | 15-27  | 15-27  | 20-23  | 19-22 | 19-23  | 19-23 | 16-24 | 19-22  | 19-22 | 19-23 |
| OP P2       | 25     | 25     | 26     | 25    | 25     | 25    | 26    | 26     | 25    | 26    |
|             | 13-38  | 19-31  | 22-27  | 24-27 | 22-27  | 22-27 | 24-27 | 24-27  | 22-28 | 21-29 |
| a-wave      | 15     | 15     | 14     | 14    | 15     | 14    | 15    | 15     | 16    | 15    |
| cone        | 15-32  | 24-36  | 10-19  | 10-18 | 15-19  | 10-18 | 13-16 | 13-16  | 14-17 | 14-17 |
| b-wave      | 30     | 30     | 31     | 31    | 31     | 30    | 31    | 31     | 31    | 31    |
| cone        | 24-36  | 24-36  | 29-34  | 28-33 | 29-33  | 27-32 | 27-33 | 27-33  | 26-34 | 26-34 |
| 30 Hz N1    | 61     | 61     | 61     | 61    | 61     | 61    | 61    | 61     | 61    | 61    |
|             | 42-80  | 42-80  | 27-84  | 30-81 | 33-89  | 36-86 | 59-62 | 59-65  | 59-62 | 58-63 |
| 30 Hz P1    | 48     | 47     | 48     | 47    | 48     | 49    | 49    | 50     | 47    | 48    |
|             | 34-59  | 35-60  | 13-64  | 18-70 | 29-79  | 23-85 | 41-50 | 43-52  | 42-47 | 37-58 |

## Discussion

The present study demonstrated the normal values of ERG responses in 55 patients with different age groups and between men and women in an eye clinic in the north of Iran. All measurements were in accordance with ISCEV guidelines (5-8). The mean difference between the ERG parameters of the present study and the study conducted by Dr. Parvaresh was 55% in the amplitudes, and 45.05% in latency time. The minimum and maximum differences were 2% and 96% in the amplitude, and 1.7% and 100% in the latency time, respectively (15). The mean difference between this study and the study conducted by Iijima was 20% in the amplitude and 18% in the latency time, and the minimum and maximum differences were 14% and 48% in the amplitude, and 2% and 43% in the latency time, respectively. The

mean difference between this study and the study conducted by Heckenlively and Arden (2006) was 47% in the amplitude and 50% in the latency time, and the minimum and maximum differences were 0% and 85% in the amplitude, and 0% and 75% in the latency time, respectively (9,12). These comparisons indicate the consistency of the results of our study with the study by Iijima (12). The possible reasons for such differences might be the racial differences, the research setting, which was a center in Tehran for Dr. Parvaresh's study with referrals from all over Iran which cannot represent a particular demographic group; the age range of 10 to 60 years in this study and 1 to 80 years in the studies by Dr. Parvaresh *et al.*, Heckenlively and Arden, and Iijima (15,9,12). Some studies such as "Birch" (14) and "Lee" (11) reported

a statistically significant difference between sexes (11-14), but we did not find a significant difference between men and women similar to the studies by Dr. Parvaresh *et al.* and Hiroyuki. In this study, only P1L, P1R, P3R, N3R, P4R and N4R had a p-value less than 0.05. However, they are clinically not important since these oscillatory potential components change under the influence of factors such as axial length, hormonal factors (1) and mood and thus they are unstable (16). There were no significant differences between age groups in terms of ERG parameters in this study, while Dr. Parvaresh, Lee, Iijima, and Birch reported reduced amplitude especially in the b-wave, and increased latency times in older subjects (over 60 years). That could be due to subtle changes of the pre-retinal layer, reduce photopigments optical density and death of Müller layer cells or bipolar disorder in the retina of older adults (15,11,12,14). It should be noted that the present study did not examine the age groups over 60. There was no significant difference between the two eyes in terms of ERG responses which is in favor of the correct placement of the electrodes on the eyes and the lack of differences in pupil sizes. It should be noted that the variable of the eye was not examined in other studies. In general, our results are consistent with other studies in different parts of the world (15,9-14) and the studies that were reviewed in this study, indicating that normal values of ERG in normal people need to be first determined accurately in every region and every ERG center before interpreting its parameters. In addition to the age range, the sample size and selection of the subjects among referrals to one center were among the limitations of this study which make the results not necessarily

indicative of the normal range of the population in that province.

## Conclusion

The results of the current study differed from other studies in terms of the values of amplitude and latency times. Therefore, it is necessary to standardize ERG for each center.

## Acknowledgment

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## Recommendations

It is recommended that a similar study with a larger sample size or a wider age range and wider refractive error range be conducted in other ERG centers in Iran to obtain the normal parameters for Iranian populations.

## Conflict of Interest

The authors have no conflict of interest.

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