Comparing Colored and White-Black Visual Evoked Potentials in Multiple Sclerosis Patients

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ABSTRACT

Introduction: We compared white-black (WB), white-red (WR), and black-red (BR) checkerboard stimulated visual evoked potentials (VEPs) in multiple sclerosis (MS) patients and aimed to evaluate if red-colored VEP is more sensitive than WB VEP for the diagnosis of optic neuritis (ON).

Methods: Twenty-nine MS patients (21 females [72.4%]) and 35 healthy control subjects (24 females [68.6%]) were included in the study. Neurological and ophthalmological examinations were conducted for all subjects and VEP and optical coherence tomography (OCT) investigations were performed.

Results: A significant difference was found between MS patients and the control group for WB, WR, BR stimulated VEP P100 latencies and retinal nerve fiber length (RNFL) and ganglion cell complex (GCC) thicknesses, but there was no difference for WB, WR, and red stimulated VEP amplitude values between the groups. There was no significant pathological difference between the eyes with an ON history in MS and eyes without an ON history in MS and control subjects after WB, WR, and BR stimulation (p=).

Conclusions: The WB checkerboard stimulated VEP is an ample test for routine use; further studies are necessary regarding the utility of red stimulated VEP in detecting subclinical ON.

Keywords: Multiple sclerosis, optic neuritis, colored visual evoked potentials, optical coherence tomography

INTRODUCTION

Multiple sclerosis (MS) is an idiopathic inflammatory demyelinating disorder of the central nervous system, and clinical presentation during the disease course may include a wide spectrum of clinical findings. Optic neuritis (ON) may be the initial presentation in approximately 20% of MS patients, and ON may occur during the course of the disease in 50% of patients with MS (1,2,3,4). The most common clinical characteristics of ON include periorbital pain mostly during extraocular eye movements, unilateral vision loss that develops over hours up to 2 weeks, and visual field defects. Patients additionally complain regarding color and contrast deficits and desaturation of bright colors, particularly red color desaturation, which has been observed in the affected patient groups not only during an attack period but also in the recovery phase (5,6,7). White-black (WB) checkerboard-stimulated visual evoked potentials (VEP) are routinely utilized for evaluating ON during an acute attack as well as for the detection of subclinical ON involvement.

In this study, we evaluated VEP findings in MS patients and looked for the sensitivity of black-red (BR) or white-red (WR) VEP and evaluated if colored VEP testing was more sensitive than white-black for detecting ON. We also aimed to determine if P100 latency correlated with other visual function parameters, such as visual acuity and optical coherence tomography (OCT).

METHODS

Patient Recruitment

In this cross-sectional, prospective study, clinically definite MS patients according to the 2010 McDonald criteria were included in the study (8). Patients had a relapsing remitting disease course and were followed up at the MS unit of Bakırköy Mazhar Osman Mental Health and Neurological Disorders Education and Training Hospital. The inclusion criteria for the study subjects were as follows: (1) subjects with no history of any systemic diseases, including diabetes mellitus and hypertension, which could affect the retina and visual pathways; (2) subjects with no history of any medications that could affect the visual pathways; (3) MS patients who were in the remittance phase; and (4) subjects who were compatible with the investigations performed. We selected MS patients according to a convenience sampling method. MS patients...
with or without ON history were grouped and healthy control subjects were evaluated, respectively. Subjects were also evaluated for mydriasis and/or anisocoria and possible mydriatic drug use in the last 24 hours prior to VEP application.

Neurological and neuro-ophthalmological exams were performed; Expanded Disability Status Scale (EDSS) scores, visual acuity (Snellen chart), and color vision (Ishihara color plates) were assessed for the patient group.

The study protocol was approved by Bakirköy Mazhar Osman Mental Health and Neurological Disorders Education and Training Hospital Ethical Committee (03.06.2014/404) and informed consent was obtained from the subjects.

**Visual Evoked Potentials Evaluation**

The study was performed in a dark and quite room in the Electrophysiology Laboratory. A Nicolet 2015 visual stimulator system was used for visual stimuli and recordings were made using a Nicolet EDX Synergy nerve conduction studies (NCS) electromyography (EMG)/evoked potential equipment, with VEP extension software at a distance of 100 cm from the screen with a checkerboard pattern of angular quantity of individual squares of 1° 17’ with 100% contrast. Visual stimuli were performed on the patients using WB, white-red (WR), and rb checkerboard stimuli using a pattern-reversal method at 1/sec frequency. Golden electrodes were used for the recordings. The active electrode was located at Oz and the reference electrode was located at Fz according to the International 10-20 System. The ground electrode was located on the right wrists of subjects. Subjects who had corrective lenses were tested with their eyeglasses. An ACER™ LCD screen sized 19 inches was used. Visual stimuli were assessed using WB, WR, and rb 12×16-piece squares with 100% contrast. Gaze was fixed at the circle in the middle of the screen, and the patient’s compatibility was carefully evaluated during the study. The results were recorded individually for each eye. At least 100 stimuli from two recordings were averaged and the stimuli number was extended to 200 when necessary. The P100 wave was identified as the first major positive peak response with a latency of approximately 100 milliseconds (ms). P100 wave latency and amplitude were assessed. The P100 latencies were recorded as ms, and peak-to-peak amplitudes were recorded as microvolts (µV). The obtained values were compared with the data obtained in the group of healthy volunteers, selected suitable in terms of age and sex.

**Ocular coherence Tomography and Retinal Nerve Fiber Layer Evaluation**

Optical coherence tomography evaluations were studied using Optovue RTVue-100 Fourier 2007, version 3.0, domain OCT at Bakirköy Dr. Sadi Konuk Hospital ophthalmology clinics.

The device is equipped with software that automatically calculates retinal nerve fiber length (RNFL) thickness in four quadrants of the visual field; the scans were analyzed using a signal quality of at least seven on a 10-point scale. OCT was performed by the same investigator blinded to the clinical evaluations of the patients. During the investigation, subjects were asked to look at the target in the device. RNFl and ganglion cell complex (GCC) research protocols were applied by optic nerve mapping for both eyes, and foveal imaging was not recorded. RNFL was evaluated using the fast RNFL thickness test. By this system, the mean nerve fiber thickness of all eyes was computed automatically in µm.

Additionally, mean GCC thickness was computed using the GCC scan protocol. According to the protocol, a 360° circular scan with the disc in the center was performed and the diameter was 3.45 mm. While evaluating the results, the cut-off value for RNFL and GCC using a receiver operating characteristic (ROC) analysis was not available and mean -2 standard deviation (SD) values for healthy subjects were accepted as pathological.

**Statistical Analysis**

The IBM Statistical Package for Social Sciences (SPSS Inc.; Chicago, IL, USA) 15.0 software was used for statistical analysis. Descriptive statistics were used for the demographic features of the cohort. The Kolmogorov-Smirnov normality test was performed for parametric data. Group comparisons were evaluated using Chi-square test; Student’s t-test was used for normally distributed parameters and the Mann-Whitney U test for parameters not normally distributed. One-way ANOVA was used to compare more than two groups. In the post hoc analyses, the least significant difference test was performed. The evaluation of non-parametric data was performed using the Chi-square test. The Pearson correlation test was used for correlation analysis. Cut-off values of P100 latencies and amplitudes of WB, WR, and rb stimulated VEP were calculated using the ROC curve. A value of p≤0.05 was considered statistically significant.

**RESULTS**

Demographic and clinical findings of the study population are shown in Table 1. There was no significant difference between the patient and control groups regarding age and gender (p=0.651 and p=0.743, respectively). A total of 58 eyes of MS patients and 70 eyes of healthy subjects were evaluated during the study.

**Visual-Evoked Potential Results**

Table 2 shows the VEP results of the study. In normal volunteers, there were significant differences between P100 latencies and amplitudes of WB, WR, and rb stimulated VEP studies (p=0.000 and p=0.000, respectively). In the post hoc analysis, although P100 latencies of the rb stimulus were found to be significantly longer than those of WB and rw stimuli, there was no significant difference between WB and rw stimuli (p=0.000, p=0.000, p=0.760, respectively).

The amplitudes of WB stimulated VEP responses were significantly higher than those of WR and rb stimuli, while there was no difference between the amplitudes of the WR and rb stimuli (p=0.000, p=0.000 and p=0.076, respectively).

Similar to healthy subjects, P100 latencies and amplitudes of VEP responses with WB, WR, and rb stimuli were found to be significantly different in MS patients (p=0.004 and p=0.000, respectively). The P100 latency of rb stimulated VEP responses was found to be longer than those of WB and rw stimuli. In contrast, there was no significant difference between WB and rw stimuli (p=0.002, p=0.007, and p=0.671, respectively). The amplitudes of WB stimulated VEP responses were higher than those of WR and rb stimuli, with no significant difference found between WR and rb stimuli (p=0.000, p=0.009, and p=0.069, respectively).

Although P100 latencies of VEP responses with WB, WR, and rb stimuli were found to be significantly longer in MS patients compared to healthy subjects (for all p=0.000), there were no significant differences of VEP amplitudes between the three different stimuli in the two groups (p=0.240, p=0.471, and p=0.104, respectively; Table 2, Figure 1, 2).

There was a strong correlation between P100 latencies and amplitudes of WB-WR, WB-rb, and WR-rb stimulated VEP responses (r=0.919, r=0.902, and r=0.939 for latencies; r=0.779, r=0.767, and r=0.712 for amplitudes, respectively). There was a negative correlation between WB, WR, and BR P100 latencies and visual acuity (p=0.224, p=0.257, and
Optical Coherence Tomography Results

Optical coherence tomography examinations were performed on 52 eyes of 26 MS patients and 40 eyes of 20 healthy subjects. Some subjects refused further evaluation and OCT data are missing. RNFL and GCC thicknesses were found to be significantly lower in MS patients than in healthy subjects (p=0.045 and p=0.016, respectively; Table 3).

There was a strong correlation between RNFL and GCC thicknesses of OCT parameters (r=0.780, p<0.001). Although not only RNFL but also GCC thickness was found to be strongly negatively correlated with P100 latencies of WB, WR, and rb stimulated VEP responses, no correlation was observed between VEP amplitudes of the three different stimuli and OCT parameters (Table 4).

Receiver Operating Characteristic Analyses

Receiver operating characteristic analyses were performed for determining cut-off values of OCT parameters and VEP responses. We could define cut-off values for P100 latencies of VEP responses but we could not define cut-off values for amplitudes of VEP responses and RNFL and GCC values of OCT examination. The rb (area under the curve [AUC]=0.923) and WB (AUC=0.919) stimulated VEP examinations showed better performance than did the WR stimuli (AUC=0.885) for P100 latencies.

The limit values that were defined by using the Youden (J) index for P100 latencies of WB, WR, and rb stimulated VEP responses were 113.5 ms (specificity, 95.7%; sensitivity, 78.4%), 111.5 ms (specificity, 84.3%; sensitivity, 81.1%), and 122.5 ms (specificity, 90%; sensitivity, 83.8%), respectively.

A history of ON was defined in 37 eyes of MS patients. The P100 latency was found to be pathological in 29 eyes (78.4%) with WB, in 30 eyes (81.1%) with WR, and in 31 eyes (83.8%) with rb stimulation, and no significant difference was found between the three different color stimuli (p=0.838). P100 latencies of VEP responses in eight eyes (38%) with WB, eight eyes (38%) with WR, and five eyes (23.8%) with rb stimulation was found pathological in 21 eyes without an ON history. There was no significant difference between the three different stimuli (p=0.526).

Table 1. Clinical and demographic findings of the study

<table>
<thead>
<tr>
<th>Age, mean±SD (years)</th>
<th>MS patients (n=29)</th>
<th>Controls (n=35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.24±9.16</td>
<td>33.31±7.14</td>
<td>0.651</td>
<td></td>
</tr>
<tr>
<td>Sex, female/male (n)</td>
<td>21/8</td>
<td>24/11</td>
<td>0.743</td>
</tr>
<tr>
<td>Disease duration, mean±SD (range) (months)</td>
<td>73.76±55.77</td>
<td>0.743</td>
<td></td>
</tr>
<tr>
<td>EDSS, mean±SD</td>
<td>1.44±1.12</td>
<td>0.743</td>
<td></td>
</tr>
<tr>
<td>ON history (+)</td>
<td>37</td>
<td>0.743</td>
<td></td>
</tr>
<tr>
<td>Visual score*, mean±SD</td>
<td>0.93±0.15</td>
<td>1±0</td>
<td></td>
</tr>
<tr>
<td>Color vision**, mean±SD</td>
<td>5.71±0.99</td>
<td>6±0</td>
<td></td>
</tr>
<tr>
<td>Fundus examination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, R/L</td>
<td>15/13</td>
<td>20/20</td>
<td></td>
</tr>
<tr>
<td>Temporal pallor, R/L</td>
<td>7/9</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Optic atrophy, R/L</td>
<td>1/1</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Total, R/L</td>
<td>23/23</td>
<td>20/20</td>
<td></td>
</tr>
</tbody>
</table>

SD: standard deviation; n: number; MS: multiple sclerosis; EDSS: Expanded Disability Status Scale; R: right; L: left
*According to Decimal Visual Acuity Scale
**According to Ishihara Test

Table 2. The results of P100 latencies and amplitudes of healthy subjects and MS patients

<table>
<thead>
<tr>
<th>Healthy subjects</th>
<th>Healthy subjects-MS patients</th>
<th>MS patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean±SD</strong></td>
<td><strong>f</strong></td>
<td><strong>p</strong></td>
</tr>
<tr>
<td>P100 WB (ms)</td>
<td>107.33±4.23</td>
<td>43.239</td>
</tr>
<tr>
<td>P100 WR (ms)</td>
<td>107.60±4.79</td>
<td>6.374</td>
</tr>
<tr>
<td>P100 rb (ms)</td>
<td>114.61±6.47</td>
<td>11.80</td>
</tr>
<tr>
<td>Amplitude WB (µV)</td>
<td>9.41±2.85</td>
<td>20.774</td>
</tr>
<tr>
<td>Amplitude WR (µV)</td>
<td>6.81±2.10</td>
<td>0.724</td>
</tr>
<tr>
<td>Amplitude rb (µV)</td>
<td>7.56±2.35</td>
<td>1.640</td>
</tr>
</tbody>
</table>

SD: standard deviation; MS: multiple sclerosis; ms: milliseconds; µV: microvolts; WB: white-black; WR: white-red; rb: black-red

Table 3. OCT results of the study

<table>
<thead>
<tr>
<th>OCT results of the study</th>
<th>MS patients</th>
<th>Healthy subjects</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNFL, mean±SD (µm)</td>
<td>103.35±15.84</td>
<td>109.55±12.56</td>
<td>0.045</td>
</tr>
<tr>
<td>GCC, mean±SD (µm)</td>
<td>94.67±10.92</td>
<td>99.48±6.59</td>
<td>0.016</td>
</tr>
</tbody>
</table>

OCT: optical coherence tomography; MS: multiple sclerosis; RNFL: retinal nerve fiber layer; GCC: ganglion cell complex; µm: micrometer

Figure 1. Visual evoked potentials latencies with three different stimuli in multiple sclerosis patients and healthy subjects

A history of ON was defined in 37 eyes of MS patients. The P100 latency was found to be pathological in 29 eyes (78.4%) with WB, in 30 eyes (81.1%) with WR, and in 31 eyes (83.8%) with rb stimulation, and no significant difference was found between the three different color stimuli (p=0.838). P100 latencies of VEP responses in eight eyes (38%) with WB, eight eyes (38%) with WR, and five eyes (23.8%) with rb stimulation was found pathological in 21 eyes without an ON history. There was no significant difference between the three different stimuli (p=0.526).
P100 latencies of three eyes (4.2%) with WB, 11 eyes (15.7%) with WR, and seven eyes (10.0%) with rb stimulation were outside of the ±2 SD limits in 70 eyes of healthy subjects. Although WB stimulation was more specific, we did not find any significant difference between the three different stimuli in healthy subjects (p=0.079).

According to these results, P100 latencies with WB, WR, and rb were found to be more pathological than RNFL and GCC thicknesses in both eyes with and without an ON history and healthy controls (p=0.000, p=0.007, and p=0.011, respectively).

**DISCUSSION**

Visual evoked potentials is a sensitive diagnostic tool for detecting visual system impairments. Axonal damage is characterized by changes in VEP amplitude, while the prolongation of latency refers to optic nerve demyelination (9). Assessing RNFL using OCT also seems to provide additional information relating to the integrity of the optic nerves (10,11). In literature, there are a few studies regarding colored VEP and their results are controversial. Although colored pattern-reverse VEP was not found to be different from WB VEP in one study, P100 latencies were found to be longer with rb, green-black, and blue-black stimuli compared to WB stimuli in another study; the authors suggested that these results might be related to greater luminance with a WB pattern (12,13,14). In these studies, colored VEP was not found to be more sensitive than WB for detecting ON (14). In our study, we also found that P100 latencies of VEP responses with rb and rw stimuli were longer than that with WB stimuli not only in MS patients but also in normal controls. Moreover, rb or rw VEP were not superior to WB VEP in MS patients for detecting ON. Accornero et al. (10) showed that the blue-black pattern-reversal VEP was more sensitive for detecting pathological responses in glaucoma suspects, but rb VEP was more sensitive in ON patients. The authors proposed that color stimuli might activate the magnocellular and parvocellular systems in different pathways. In a more recent study, the authors studied rb and blue-black checkerboard stimuli (isocontrast VEP) and compared them with pure chromatic contrast with red-green and blue-yellow grading standard color VEP. In this study, two different techniques were found useful for detecting and differentiating mild vision disorders (15). In another study, low-contrast stimulation VEP was investigated in MS patients, and it was found that prolongation of VEP latency correlated with visual acuity. They suggested that the magnocellular system might be related to achromatic low-contrast stimuli. In contrast, in a recent review, Skottun reported that the relationship between VEP responses and magnocellular and parvocellular pathways might be over exaggerated (16).

In our study, different color patterns were utilized to determine if there was a difference between color patterns in terms of sensitivity and specificity. While evaluating and diagnosing ON in unaffected eyes, measurements of both the VEP amplitude and the RNFL thickness can be valuable. These evaluations seem to provide an insight into the axonal integrity in MS patients even without a history of ON. Both OCT and VEP are useful methods providing different but complimentary information regarding the optic nerves. When the symptoms of ON are not clear or the patient cannot be evaluated adequately due to cognitive impairment, OCT and VEP provide further data for diagnosing ON.

The most common method for VEP involves high-contrast pattern-reversal black/white checkerboards displayed at differing spatial frequencies. This method seems to activate many components of the visual system and create a large cortical response. Recent studies of VEPs have focused on different methods to assess the activity of different and specific components and evaluate various visual impairments. A recent study suggested that achromatic stimuli (especially low-contrast gray/black checkerboards) could detect visual impairment even in mild stages of ON, but failed to differentiate ON and glaucoma (10). Conversely, chromatic patterns managed to differentiate ON from glaucoma, with the red/black pattern responses being slower in ON patients and the blue/black pattern responses slower in patients with glaucoma. Recent literature reported red dyschromatopsia in ON and thus color evaluation seems to be subclinically impaired even in the earliest stages of ON. In this study, it was suggested that a color/black low-contrast pattern-reversal checkerboard stimulation procedure could evoke robust and stable VEPs suitable for routine clinical testing by activating distinct components of the visual pathway and allowing a better definition of the visual impairment in different pathologies, even at the very early stages of the disease (10).

Recent literature seems to support utilizing both VEP and OCT while evaluating ON patients, and an equally strong correlation was found between the mean VEP amplitude and RNFL thickness, both in the eyes with and without a history of ON (17). This study indicated a correlation between the reduced VEP amplitude and decreased RNFL thickness in patients with a history of ON. However, contrary to previously reports, the authors found that VEP is more sensitive than OCT examination in eyes affected and unaffected by ON. They suggested that the anterior visual pathway could be evaluated during OCT examination, but that VEP examination could give information regarding the integrity of both anterior and posterior visual pathways. Moreover, it considered that after an...
ON episode, axonal damage could be secondary to demyelination, but the reduction in the RNFL thickness would proceed retrograde and might not always be complete. Recently, several suggestions to improve the sensitivity of OCT have been offered, and in addition to RNFL thickness, the measurements of the neuronal ganglion cell layer and the inner plexiform layer would provide additional data (17). In addition to visual acuity, visual field, and color defects, abnormal contrast vision is also a feature of demyelinating optic neuropathy which is commonly observed in patients with MS even without a history of ON. In another study, the spectrum of visual dysfunction in MS patients was evaluated using high- and low-contrast pattern-reversal checkerboard stimulation VEP and latencies of low-contrast stimuli were significantly increased in MS patients without ON history when compared to controls. VEP findings also correlated with RNFL thickness.

Recent literature shows the significance of evaluating VEP latency and amplitude for detecting both clinical and subclinical optic nerve involvement. If a single method for the approval of an ON history is required, VEP should be preferred. However, the combination of the VEP and OCT methods would facilitate further insight for ON (16). In conclusion, WR or BR stimulation VEP does not seem to be superior to WB stimulation VEP. It should also be noted that while rb VEP has a low specificity for differential diagnosis of ON, this method may help to identify a contralateral subclinical optic neuropathy in patients with their first episode of acute idiopathic ON who have few or no white matter lesions on magnetic resonance imaging because of its high sensitivity rates (83.8% in MS patients). This method might also prove useful for monitoring disease progression and response to treatment in selected patients. Further long-term studies are warranted involving the utility of pattern-reversal VEP and OCT in patients with ON.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Bakırköy Prof. Mazhar Osman Psychiatry and Neurological Disorders Hospital Ethical Committee (03.06.2014/404).

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.


**Conflict of Interest:** No conflict of interest was declared by the authors.

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