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Research Article

Retinal Function Assessment in Alcohol Use Disorder Patients

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Abstract

Objective. Retinal injury induced by ethanol consumption has been previously reported in animal models, including biochemical, histological and functional alterations. These results need to be clinically tested in alcoholic patients which do not report several systemic or ophthalmic diseases. *Methods.* Six patients with alcohol use disorder were recruited from an 'Alcoholism Treatment Unit'. All of them with active alcohol consumption when the study was conducted or that had stopped drinking six months prior to the study, with no ocular disease or visual acuity alterations. All patients underwent fundus photography, optical coherence tomography (OCT) as well as visual field test. Electrophysiological tests were conducted to check retinal function: Ganzfeld Electroretinogram (ERG), Pattern Electroretinogram (PERG), Multifocal Electroretinogram (mfERG), and also Visual Evoked Potential (VEP). *Results.* Visual acuity was normal in all cases as well as fundus photography and visual field test. The OCT showed a mild decrease in the retinal nerve fiber layer thickness average in three patients. Five patients showed impairments in mfERG response, decreased amplitude in ERG response and no significant alterations in PERG and VEP. *Conclusion.* Although standard ophthalmic tests did not show signs of an ocular disease, the study of electrical function showed different impairments in almost all patients. The alterations reported in mfERG and ERG recordings could reflect inner retina injury, thus supporting the possible existence of an alcoholic retinopathy. Further studies with larger number of subjects are necessary to assess the specific impact of other factors such as tobacco or nutritional status on patients with alcohol use disorder.

Keywords: alcohol, electroretinogram, visual evoked potential

1. Introduction

Alcohol is a psychoactive substance with dependence-producing properties. As described in The World Health Organization (WHO) "Global Status Report on Alcohol and Health 2014", consumption of and problems related to alcohol vary widely around the world, but the burden of disease and death remains significant in most countries. The harmful use of alcohol ranks among the top five risk factors for disease, disability and death throughout the world; it is a causal factor in more than 200 disease and injury conditions (and has also serious social and economic consequences for individuals other than the drinker, and for society at large).

Ethyl alcohol (ethanol) consumption has a high prevalence in our study population, at least 84.4% for men and 72.7% for women between 15 and 64 years old consumed alcohol in the last month [1]; chronic consumption is also important, between 4.7 and 13% of the population in different studies [2,3], although this is not normally recognized in the surveys.

Alcohol-related peripheral neuropathy (ALN) is a potentially debilitating complication of alcoholism that results in sensory, motor, and autonomic dysfunction [4], which initially was widely assumed to primarily reflect consequences of nutritional deficiency [5]. In recent studies, failure of thiamine treatment to reverse ALN, together with new information demonstrating clinical and electrophysiological distinctions between ALN and nutritional deficiency neuropathies, suggests that alcohol itself may significantly predispose and enhance development of neuropathy in the appropriate clinical setting [6,7,8,9]. Thus, ethanol exerts its deleterious effects metabolically via oxidative and nonoxidative

pathways [10], involving free radical production and lipid peroxidation [11], potentially leading to an imbalance between oxidants and antioxidants in favor of the former, resulting in an increased oxidative stress [12].

In the visual system, it is known that long-term ethanol consumption causes clinical manifestations that were also attributed to nutritional deficits rather than a direct effect of ethanol [13,14]. Increasing evidence suggest that oxidative stress contributes to the pathogenesis of many ocular neurodegenerative disorders, such as diabetic retinopathy [15], age-related macular degeneration [16] or uveitis [17,18,19,20]. Also our research group has previously reported that chronic alcohol consumption induces oxidative stress in rat optic nerves [7], which seems to mediate direct ethanol toxicity related to oxidative stress, discarding the influence of the nutritional status on the parameters studied.

The retina, which is the neurosensorial ocular tissue, is extremely rich in membranes with polyunsaturated lipids [21], making it particularly sensitive to oxygen free radicals and lipid peroxidation [22]. Although alcoholic retinopathy has not yet been described as a disease and no defining diagnostic criteria exist at the moment, our research group has previously reported ethanol-induced biochemical, histological and functional alterations in rats [8,23]. These results need to be clinically tested in alcoholic patients without ophthalmic disease, being this issue the main objective of our study.

2. Methods

Data were available for six alcoholic patients, all of them active users of alcohol when the study was conducted or that had stopped drinking six months prior to the study. Subjects were free of ocular disease or any visual acuity alterations. There was no severe systemic disease, mainly hypertension, diabetes mellitus or liver disease. Diagnosis was established clinically by a retina specialist and confirmed by visual acuity (measured following the guidelines from Early Treatment Retinopathy Study (EDTRS), 1985) [24], fundus photography, optical coherence tomography (OCT) and visual field test.

Fundus photography was obtained with a TRC-50IX-Retinal Camera from TOPCON®, with a MY-10 camera and IMAGEnet 2000 v.2.59 imaging software also from TOPCON®.

OCT was recorded with a 3D-OCT-2000 equipment from TOPCON®. The evaluation of the optic nerve consisted of the measurement of average retinal nerve fiber layer (RNFL) thickness, as well as an interpretation of the spatial distribution in quadrants and clock-hours.

Visual field data were acquired in both eyes by campimetry using a Humphrey FA II Visual Field Analyzer (Carl Zeiss, Inc. San Leandro, CA USA).

Once ocular disease was discarded, electrophysiological tests were conducted to test retinal function. Thus, all patients also underwent a Ganzfeld Electroretinogram (ERG), Pattern Electroretinogram (PERG), a Multifocal Electroretinogram (mfERG) and Visual Evoke Potential (VEP). Updated standards of the International Society for Clinical Electrophysiology of Vision (ISCEV) [25,26,27,28,29,30,31] were followed to obtain all recordings, using a Roland Consult® equipment with the application software RETIscan 3.20/RETIport 32. The procedures were as follows:

VEP recording was carried out by stimulating the patient with a Pattern reversal displayed on screen width 8.5º and stimuli squares spanned at a visual angle of 0.5º (30 min). No pupil dilation was induced in the patient and the necessary refraction was used to fix the central point in the screen, indicated by a red cross. The active electrode was placed two centimetres above the inion on the medium line (OZ) according to the international system 10-20. After that, a reference electrode was placed on the forehead (Fz) and a third electrode at Cz level as earth electrode. The impedance of the electrodes was kept under 5kΩ (with less than 20% difference between electrodes) using abrasive paste Everi® from Spes Médica (Gennova, Italy) to clean the skin, applied with sterile gauze pads. The surface electrodes were filled with conductive paste Ten20®Conductive and were fixed on the skin with Micropore™ Hipoalergenic tape from 3M (Minnesota, USA). Both eyes were stimulated separately and a maximum of 100 responses were averaged for each eye until a stable wave shape was reached. The study was repeated in a retest to ensure a reproducible response, and changes in the latency and amplitude of P100 wave (P100 latency and P100-N135 amplitude) were assessed.

PERG was carried out by stimulating the patient with a Pattern reversal, keeping the width of the screen at a visual angle of 8.5º and the size of the stimulus squares at 1º (60 min). The active HK-Loop electrodes were placed inside the conjunctival sac to prevent vision interferences. The surface reference electrodes were located on the external edge of each eye, and the ground electrode was placed on the forehead. Impedance in electrodes was kept at the same values as in VEP recording. Both eyes were simultaneously stimulated and a maximum of 200 responses averaged for each eye until a stable wave shape was reached. Changes in latency and amplitude of P50 wave (P50 latency and P50-N95 amplitude) were assessed.

mfERG was carried out with a CRT monitor with a central fixation point marked with a X across the entire screen. The stimulus array consisted of 103 hexagons which change from black to white in a pseudo-random way. The patient was placed with a fixation angle on the screen of 30 min with the head resting on an adjustable chin cup, and a minimum of 8 exploration cycles (at least one minute each) and a maximum of 12 (until responses that could be assessed were obtained) were displayed. The same electrodes as in the PERG were used. Pupils

had been dilated with pupil diameter of at least 7 mm. Refraction was corrected with lenses enabling the patients to fixate the screen properly.

The changes observed in the mfERG evaluated the central 30º of the retina following a standard distribution in rings and quadrants. Rings were numbered 1 to 6, number 1 being the central, and number 6 the most peripheral. Ring 1 corresponded to an area of 0 to 3 deg²; ring 2 to 13 deg², ring 3 to 18 deg², ring 4 to 25 deg², ring 5 to 32 deg² and ring 6 to 40 deg². Quadrant 1 consisted of the average response of the mfERG from the upper right quadrant of retinal view; quadrant 2 from the lower right; quadrant 3 from the lower left; and quadrant 4 from the upper left. Each of these response averages had an area of 32 deg².

Ganzfeld ERG was carried out with maximal pupil dilation by application of drops of Tropicamida (Alcon Cusí, El Masnou, Barcelona) and Fenilefrina (Alcon Cusí, El Masnou, Barcelona), until pupil diameter reached 7 mm in size. Electrodes were placed as in PERG.

Stimulus was presented using a Ganzfeld dome (Q450HF RolandConsult®) as recommends ISCEV to allow the control of stimuli diffusion as well as the background light. A high-pass 1Hz filter and low-pass 300Hz were used.

The procedure was as follows:

1. Scotopic conditions: recorded after at least 25 minutes of dark adaptation.

1.1. Rod response: Stimulated by a low intensity (0.01 cd_s_m-2) and frequency (0,2 Hz) flash.

1.2. Mixed response: Stimulated by a high intensity (3.0 cd_s_m-2) and low frequency (0,3 Hz) flash. This ERG response is a mixed rod-cone response, being therefore the maximum amplitude recording.

1.3. Oscillatory potentials: we used the same conditions as in the mixed response but using a frequency filter (high-pass 100Hz filter and low-pass 500Hz) which magnify the amplitude wavelets on the ascending limb of the b-wave. High intensity (3.0 cd_s_m-2) and low frequency (0,1 Hz) were used.

2. Photopic conditions: recorded after at least 10 minutes of light adaptation by steady light backgrounds (30 cd_m-2). Under this condition rod response is suppressed, and only cone response is recorded.

2.2. Cone response: Stimulated by a high intensity (3.0 cd_s_m-2) and low frequency (0,2 Hz) flash.

2.3. Flicker response. Stimulated by a high intensity (3.0 cd_s_m-2) and high frequency (30 Hz) flash, to study the macular cone response.

b-wave amplitude was measured in rods, cones and mixed response. The amplitude of the oscillatory potentials was taken as the sum of the amplitudes of the four peaks. Flicker response was evaluated by measuring the second-harmonic amplitude.

3. Results

All the patients were men with an average age of 42,6 being the younger 37 and the older 54 years old. Visual acuity was normal in all cases as well as fundus photography and visual field test.

The OCT showed no significant changes in the retinal nerve fiber layer thickness average although a mild decrease was observed in three patients (Figure 1). Measurable alterations in the different retinal layers were not observed either.

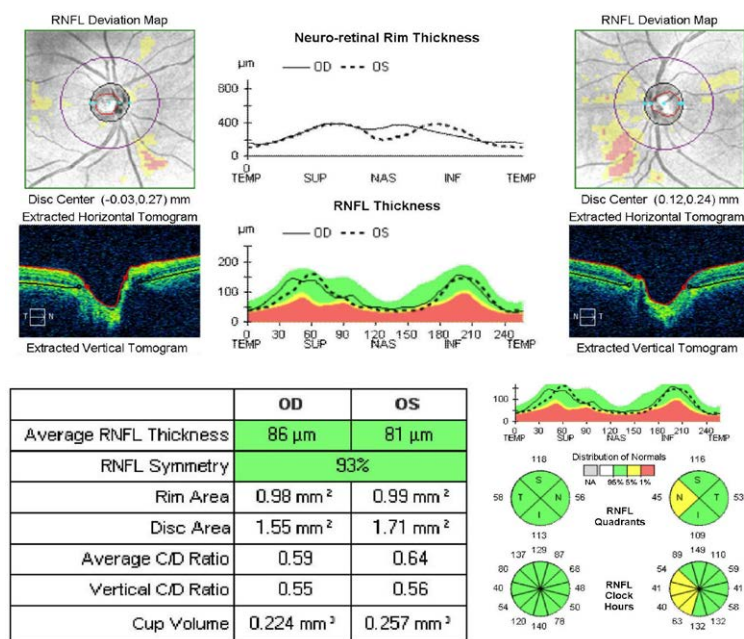No significant changes were observed in VEP or PERG.

	OD	OS
Average RNFL Thickness	86 µm	81 µm
RNFL Symmetry	93%	
Rim Area	0.98 mm²	0.99 mm²
Disc Area	1.55 mm²	1.71 mm²
Average C/D Ratio	0.59	0.64
Vertical C/D Ratio	0.55	0.56
Cup Volume	0.224 mm³	0.257 mm³

Figure 1: OCT study: Recording obtained from one of the patients showing mild alterations in the nasal quadrant, but without changes in the average of the retinal nerve fiber layer thickness.

Regarding electrophysiological tests, five patients showed abnormal responses (Table 1). In these five patients several alterations were observed in mfERG. Thus, alterations in the upper half field (mainly in the periphery) were observed in four patients (Figure 2). In one of them the alteration was observed in the temporal half field of both eyes while in another patient the impairment was observed in the nasal half field of both eyes. All the impairments were observed in the periphery, central areas were not affected.

In Ganzfeld ERG a mild decrease of b wave amplitude was registered in four patients. All of them showed an impairment of the mixed response although only in one of them was monocular. The ratio b/a was inverted in one of the patients (Figure 3). Two patients showed a decrease in rod response in both eyes and in cones response in one eye. Only one patient showed all ERG responses altered.

4. Discussion

Standard ophthalmic tests did not show signs of an ocular disease in alcoholic patients, since visual acuity was normal in all cases as well as fundus photography and visual field test. Although the OCT showed mild alterations in the retinal nerve fiber layer thickness in some patients, these findings are not enough to confirm any ocular disease.

However, when electrical function was studied, different alterations were observed in almost all patients, thus indicating that alcohol toxicity also affects the retina. It has been documented that electrophysiological tests are very sensitive to detect early stages of retinal degenerative disorders [32,33], therefore the alterations observed in these alcoholic patients could be the first step to assess the existence of an alcoholic retinopathy.

Table 1: Relevant parameters of ERG from study subjects. ERG results: N: Normal; Pat: pathological; G-ERG: Ganzfeld-ERG; G-ERG b/a: b/a ratio; P-VEP: Pattern VEP; P-ERG: Pattern-ERG; AMP: amplitude; LAT: latency; MF-ERG: Multifocal-ERG.

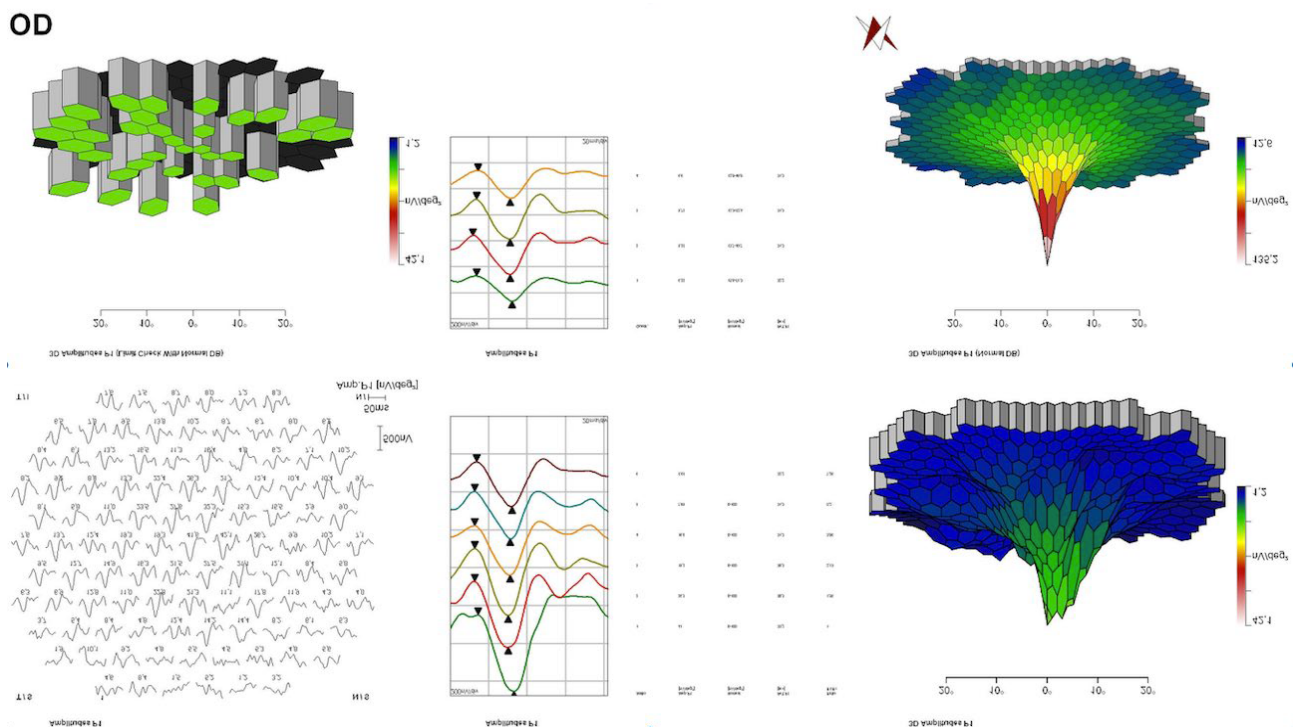| Patient	Eye	G-ERG	G-ERG b/a	P-VEP AMP	P-VEP LAT	P-ERG AMP	P-ERG LAT	MF-ERG
1	Right	N	N	N	N	N	N	N
	Left	N	N	N	N	N	N	N
2	Right	N	N	N	N	N	N	Pat
	Left	N	N	N	N	N	N	Pat
3	Right	Pat	N	N	N	N	N	Pat
	Left	N	N	N	N	N	N	Pat
4	Right	Pat	Pat	N	Pat	N	N	Pat
	Left	Pat	Pat	N	Pat	N	N	Pat
5	Right	Pat	N	N	N	N	N	Pat
	Left	Pat	N	N	N	N	N	Pat
6	Right	Pat	N	N	N	Pat	N	Pat
	Left	Pat	N	N	N	N	N	Pat

Figure 2: MF-ERG response: peripheral response shows a mild decrease in the upper half field.
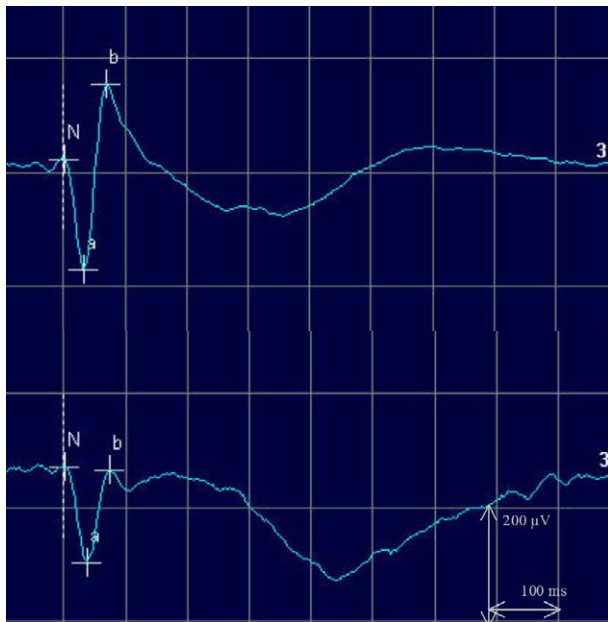
Figure 3: Ganzfeld-ERG Mixed response: normal response (top), inverted b/a ratio (bottom). Average of 3 measurements.

No changes in VEP were observed since visual acuity was normal in all cases, and VEP is only affected when the central 10° of the retina are altered. However, this technique is necessary when visual acuity is altered since alcoholic neuropathy can affect the optic nerve [5]. Moreover, concomitant consumption of alcohol and tobacco can induce optic neuritis [34].

PERG neither revealed pathological findings. This is not surprising because no signs of central retinal injury were observed, and PERG is sensitive to macular damage. Moreover, PERG is also impaired when ganglion cells are damaged and there are no signs that suggest optic neuropathy.

mfERG, which evaluates the central 30º of the retina following a distribution in rings and quadrants, showed a decrease of the response in the upper half fields, mainly in the periphery. These alterations were mild and showed an irregular pattern. No impairments were observed in the central region of the retina. Such alteration of responses in peripheral retina is also observed in other toxic retinopathies as produced by DDI (dideoxyinosine) [35] or vigabratrin, which produces affectation in both inner and external retina [36,37].

ERG measures the stimulation of the entire retina. We report an alteration of the mixed response, thus confirming the existence of a retinal injury in alcoholic patients. There are no results indicating exclusive photoreceptor cellular damage, since cones and rods responses are normal. One patient showed an inverted b/a ratio which is a common finding in diseases such as X-linked juvenile retinoschisis, congenital stationary night blindness, central retinal artery occlusion, birdshot chorioretinopathy or melanoma-associated retinopathy [38], and reflects inner retinal dysfunction. Fundus

examination is rarely diagnosed in these disorders and therefore, careful electrophysiological assessment of retinal function is needed for accurate diagnosis [39]. It is remarkable that only one patient showed this kind of impairment, being the most frequent event in this study the decrease of b wave amplitude, but without b/a wave ratio inversion.

Although 'alcoholic retinopathy' has not yet been described as a disease and no defining diagnostic criteria exist at the moment, our research group has previously reported injury induced by ethanol consumption showing biochemical, histological and functional alterations in rat retina [8,23]. In the present study carried out in alcoholic patients, standard ophthalmic tests did not show signs of ocular disease. However, when electrical function was studied, different impairments were observed in almost all patients. The alterations reported in mfERG and ERG recordings could reflect inner retina injury, thus supporting the possible existence of a real 'alcoholic retinopathy'. Unfortunately, the number of patients is insufficient to conclude that alcoholic retinopathy exists in humans. Only six patients in Alcoholism Treatment Unit were eligible to enter the study since alcoholic patients usually have systemic diseases associated with their disease that can affect the responses in the visual pathway. In addition, it must be considered that many patients have nutritional and deficiency alterations that can also cause damage in the retina. Further studies with a bigger number of subjects are necessary to confirm the results herein and assess the impact of other factors such as tobacco or nutritional status.

5. Acknowledgments

6. Conflict of interest

The authors declare that there are no conflicts of interest.

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