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Supernormal Electro-Oculograms in Patients with Neurofibromatosis Type 1

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Abstract

Purpose: To asses the retinal pigment epithelium (RPE) function measured by EOG testing in patients with neurofibromatosis type 1 (NF-1). Our preliminary EOG results suggested dysfunction of the RPE in individuals with NF-1. In order to confirm our initial results we performed EOG examination on a larger group of NF-1 patients.
Patients: Studies were performed on 36 patients with clinically diagnosed NF-1 and compared to normal healthy controls.
Methods: Standard EOG recordings were performed in accordance with the International Society for Clinical Electrophysiology of Vision (ISCEV) standards.
Results: In NF-1 patients the Arden indexes of the EOG test were significantly higher primarily due to the lower values of dark troughs. Supernormal EOGs (exceeding the value of the mean + 2 SD from the control group) were present in 58% of NF-1 patients.
Conclusions: Dysfunction of the RPE is a characteristic feature of individuals with NF-1.

Introduction

Neurofibromatosis type 1 (NF-1), or von Recklinghausen disease is one of the most common genetic multisystem progressive disorders with an incidence of approximately 1:3000 live births. In the eye, the disorder is characterised by Lisch nodules, optic gliomas, choroidal hamartomas and congenital hypertrophy of the retinal pigment epithelium (CHRPE) [1, 2]. The gene for NF-1 is localized to chromosome 17q11.2. and its protein product has been termed neurofibromin [3, 4]. It appears that reduction of neurofibromin expression can lead to abnormalities in the differentiation and migration of melanoblasts and melanocytes which gives rise to characteristic café-au-lait spots of the skin [5]. Café-au-lait spots are characterised by increased levels of epidermal melanogenesis [6] and increased numbers of epidermal melanocytes [7] which contain abnormally large pigment granules, known as macromelanosomes. In the eye, Lisch nodules consist of masses of melanocytes. The choroidal hamartoma is similar to the iris lesion histopathologically. Congenital hypertrophy of retinal pigment epithelium, a rare feature of patients with NF-1, consists of focal areas of pigment epithelial cells that are more densely packed with pigmented granules [8].

Retinal pigment epithelium (RPE) also contains melanin. It can be hypothesized that changes in neurofibromin expression are leading to some melanin changes also in RPE of patients with NF-1. It has been described in patients with albinism that the reduced level or absence of retinal pigment is associated with changes detectable by EOG [9] – the most commonly used electrophysiological test of RPE function [10, 11].

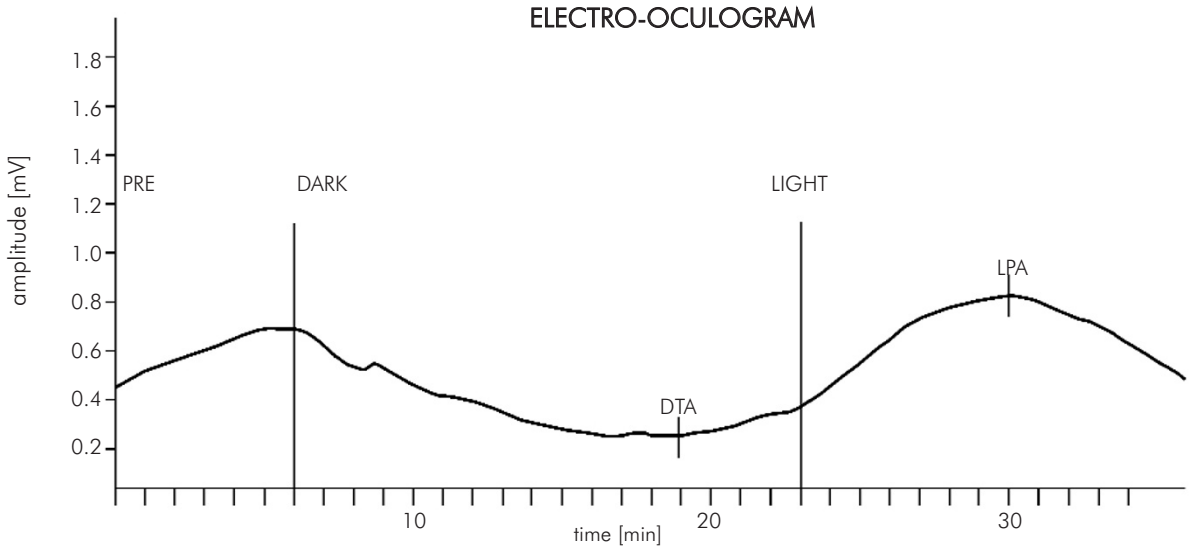
Our initial EOG [12] results suggested that dysfunction of the RPE may be a characteristic feature of individuals with NF-1. In order to verify our preliminary findings we performed the EOG examination in a two-fold larger group of patients diagnosed with NF-1.

Material and methods

The EOGs were performed on 36 patients (67 eyes; 22 males, 14 females; mean age: 26.6±10.9 years; mean refractive error: -0.21±0.72 D) who fulfilled the National Institutes of Health clinical diagnostic criteria for NF-1 [7, 13] and compared to 32 healthy subjects. Ocular findings in a group of patients with NF-1 are as follows: Snellen visual acuity – 20/20, Lisch nodules – (65/67) 97% of analysed eyes, choroidal hamartoma – (5/67) 7.4%, CHRPE (congenital hypertrophy of the retinal pigment epithelium) – (4/67) 5.9%, optic nerve glioma – (3/67) 4.4%, normal colour vision and visual field measured by kinetic perimetry. The two groups were similar as far as age, sex and refractive error were concerned. Uncooperative patients were excluded from the study.

Electro-oculography (UTAS E-2000) was performed according to the Standard for EOGs of the International Society for Clinical Electrophysiology of Vision (ISCEV) Standardisation Committee [10, 11]. In the EOG examination, patients' pupils were dilated (10% neo-synephrine, 1% tropicamide) and a stimulus intensity equal to ca. 100 $cd \cdot m^{-2}$ (aperture 1/2) was used, which has been recommended as a standard for the light adaptation phase in this case. We analysed the values of the lowest potential reached during the dark phase (dark-trough amplitude in μV, DTA), dark-trough latency (DTL) measured in minutes, the highest potential reached during light exposure (light-peak amplitude in μV, LPA), light-peak latency (LPL) in minutes and the ratio of the light-peak amplitude to dark-trough amplitude (Arden Index, AI).

Statistical analysis was performed using parametric (Shapiro-Wilk test, Student t-test) and non-parametric (Mann-Whitney test) tests with a significance level of $p \leq 0.05$. This study was approved by the Committee of Medical Ethics of the Medical University in Szczecin.



	LATENCY	AMPLITUDE	μV/DEG
Dark Trough:	13 min.	0.211 mV	7.03
Light Peak:	30 min.	0.826 mV	27.54
Arden Ratio:	3.92		

Fig. 1. Supernormal AI on EOG examination of a NF-1 patient

Table 1. EOG in NF-1 patients - descriptive statistics and statistical analysis (36 patients)

	n	N	M ± SD	Min	Med	Max
NORM- DTA (μV)	64	+	408.1±112.9***	198	403	798
NF-1- DTA (μV)	67	+	305.0±81.2***	140	296	498
NORM- DTL (min)	64	-	11.7±2.0	6	12	15
NF-1- DTL (min)	67	-	11.6±2.2	7	12	15
NORM- LPA (μV)	64	+	982±242	445	951	1650
NF-1- LPA (μV)	67	-	971±229	537	922	1483
NORM- LPL (min)	64	-	8.4±1.3***	6	8	11
NF-1- LPL (min)	67	-	7.6±1.1***	5	7	10
NORM- AI	64	+	2.45±0.37***	1.83	2.42	3.26
NF-1- AI	67	+	3.42±0.62***	2.19	3.17	5.18

n – number of eyes; M – arithmetic mean; Min – minimum value; Med – median; N – normal distribution; SD – standard deviation; Max – maximum value
**** – p<0.001*

Results

Descriptive statistics and statistical analysis of the EOG parameters for two study groups (NF-1 patients and normal controls) are shown in Table 1. The Student t-test and the Mann-Whitney test revealed significant differences in the variables DTA ($p<0.001$), AI ($p<0.001$), and LPL ($p<0.001$). Figure 1 illustrates an example of an NF-1 patient with supernormal AI on EOG examination.

For the NF-1 group, the mean DTA (305.0±81.2 μV) was significantly lower than that of the control group (408.1±112.9 μV) and the mean AI (3.42±0.62) was significantly higher (2.45±0.37). The mean LPL for the NF-1 group (7.6±1.1 min) was significantly lower than that of the control group (8.4±1.3 min). The mean LPA of the NF-1 group (971±229 μV) was lower than that of the normal subjects (982±242 μV); this difference was not statistically significant. The mean DTL for the NF-1 group (11.6±2.2 min) was almost the same as that of the control group (11.7±2.0 min).

In the group of patients with NF-1 we found no subnormal EOGs. Supernormal EOGs (AI>mean+2SD – more than 3.19) were detected in 58.3% (21/36) of patients, 58.2% (34/67) of eyes.

Discussion

The results of our studies suggest that dysfunction of RPE as measured by EOG is present in patients with NF-1. Enlargement of the analysed eyes of patients with NF-1 (from 35 to 67 eyes) did not change significantly the EOG results obtained in a group of patients with NF-1 published previously [12]. We observed a supernormal EOG (increased AIs) in 58% of analyzed eyes in individuals with NF-1. Subnormal EOG was not found in this group of patients. Detection of changes by EOG and in parallel small changes in ERG what was shown previously [12] indicating that the occurrence of abnormalities was mainly in the RPE.

The increased AI in NF-1 patients is a result of relatively low DTAs. The dark-trough potential is a result of polarisation differences between the apical and basal membranes of RPE cells [13]. The significantly lower amplitude of this potential suggests that the mechanisms underlying polarization differences are altered in NF-1.

The apical membrane is more hyperpolarized than the basolateral membrane. This variation in membrane potentials results from differences in the types and distribution of transport mechanisms between the two RPE membranes. Primarily K^+ channels generate the apical membrane potential with smaller contributions from the electrogenic Na^+/K^+ pump and $Na^+HCO_3^-$ cotransporter. The basolateral membrane potential is generated primarily by a balance between Cl^- and K^+ channels [13]. The basolateral membrane Cl^- conductance may be modulated by intracellular Ca^{2+} [14]. It is reasonable to expect that changes in any of above transport mechanisms can occur in NF-1.

As mentioned in the introduction there are data indicating a correlation between the occurrence of neurofibromin and melanin abnormalities. Melanin has the capacity to bind and accumulate many chemicals [15]. Pigmented cells contain particularly high amounts

of calcium, reflecting the enormous calcium binding capacity of melanin [16]. The data obtained in the current study suggest that supernormal EOGs in NF-1 patients are a result of changes in calcium levels caused by melanin abnormalities, which is related to the reduction in expression of neurofibromin.

The lower EOG dark-trough values have been described previously in albinism [9]. This disorder is characterised by congenital reduction or total absence of pigment in hair, skin and eyes [17].

This finding also supports the hypothesis that EOG changes in NF-1 patients are associated to neurofibromin-mediated melanin alterations.

It is well known that in many ocular disorders electrophysiological changes can occur independently of alterations detectable by ophthalmoscopy. Also in our patients abnormal EOGs were observed in individuals without characteristic NF-1 fundus changes detectable during routine examination.

NF-1 can be unequivocally diagnosed by the detection of NF-1 gene mutations by DNA/RNA analyses. Molecular analysis of large genes like NF-1 are still complex, time consuming and expensive. Due to cost implications genetic analysis of NF-1 has to be limited to the groups of pre-selected patients that have a high probability of carrying mutations. In order to identify such groups it is important to find as many independent clinical features as possible that are closely correlated with NF-1 disease expression.

The high frequency of supernormal EOGs in patients with NF-1 suggests that this type of analysis might be useful in the initial identification of patients with NF-1 who do not present with the typical spectrum of disease symptoms.

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