ALTERATIONS IN NOCTURNAL MELATONIN LEVELS
IN PATIENTS WITH OPTIC NEUROPATHIES

ALTERACIONES DE LA SECRECIÓN NOCTURNA
DE MELATONINA Y NEUROPATÍAS ÓPTICAS

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ABSTRACT

Objective: To study nocturnal melatonin suppression induced by exposure to light in patients with bilateral optic neuropathies.

Methods: Observational, prospective case control study. Twenty patients were included in this study and distributed in 3 groups: Group A (n=5, Healthy Control Subjects), Group B (n=10, Experimental Patients) and Group C (n=5, Blind Control Subjects). LogMAR best-corrected visual acuity, standard automated perimetry mean deviation, retinal nerve fiber layer thickness by Optical Coherence Tomography and multifocal electroretinography (mfERG) were used to evaluate the changes. Melatonin was analysed in the saliva by radioimmunoassay after exposure to light (600 lux for 1 hour) (nocturnal melatonin suppression test).

Results: Statistically significant differences between the groups were found. No changes in the mfERG results were detected. The nocturnal melatonin suppression test was positive in all cases in Group A, 50 % in Group B and none in Group C.

RESUMEN

Objetivo: Evaluar la supresión de la secreción nocturna de melatonina inducida por exposición a la luz en pacientes con neuropatías ópticas bilaterales.

Métodos: Estudio clínico de casos controles, observacional y prospectivo. Tamaño muestral de 20 pacientes distribuidos en 3 grupos: Grupo A (n=5, Sujetos Sanos Controles), Grupo B (n=10, Pacientes Experimentales) y Grupo C (n=5, Sujetos Controles Ciegos). Se analiza la mejor agudeza visual corregida LogMAR, la desviación media en perimetria estática automatizada, el espesor medio de la capa de fibras nerviosas retinianas mediante Tomografía de Coherencia Óptica y los registros de electroretinografía multifocal (mfERG). Se realizan determinaciones de melatonina en saliva por radioinmunoesay tras exposición a una luz de 600 lux durante 1 hora (Test de supresión nocturna de melatonina).

Resultados: Se encontraron diferencias estadísticamente significativas entre los grupos. No se observaron cambios en los registros de mfERG. El test de
Conclusions: Half of the patients with optic neuropathies and severe visual loss were shown to suffer significant melatonin regulation anomalies, probably due to the dysfunction of the intrinsically photosensitive retinal ganglion cells (ipRGC) (Arch Soc Esp Oftalmol 2009; 84: 251-258).

Key words: Melatonin, circadian rhythms, supra-chiasmatic nuclei, light effects, optic neuropathies.

INTRODUCTION

Melatonin is a synthesized hormone segregated by the pineal gland with circadian periodicity (1). The light, phototransduced in the intrinsically light-sensitive retina ganglion cells (ip RGC) (2,3), is conducted through the retina-hypothalamus tract (TRH) to the supra-chiasmatic nucleus of the hypothalamus (SCN), the main mammal circadian clock (4) that connects the retina with the pineal gland through a multi-synaptic neural pathway (5).

Light has two effects on melatonin secretion (6): 1) the light-darkness cycles synchronizes the circadian rhythm of its secretion, 2) light of sufficient intensity and duration brusquely inhibits its secretion in a dosage dependent form.

In humans, plasmatic melatonin is practically of pineal origin, with the hormone entering the bloodstream due to passive diffusion (7). Its secretion increases soon after nightfall and peaks in the middle of the night between two and four a.m., gradually decreasing in the second half of the night (7). The concentrations of melatonin in saliva are approximately 30% of plasmatic levels, exhibiting the same circadian rhythm. There is a perfect correlation between pineal, plasmatic, urinary and salivary melatonin (8,9).

In this paper the aim is to assess the functional integrity of RHT and the neuroendocrine response of the pineal gland to light in patients with severe bilateral optic neuropathies by means of establishing the melatonin in saliva through radio immunoassay (RIA). In this regard, we related the suppression of the nocturnal secretion of melatonin induced by exposure to a strong light of 600 lux during one day with the severity of the loss of visual function caused by damaged retinal ganglionary cells. The severity of the optic neuropathy is to be documented and confirmed by means of morphological diagnostic techniques such as Optic Coherence Tomography (OCT) and functional techniques such as multifocal electro-retinography (mf ERG).

SUBJECTS, MATERIAL AND METHODS

Subjects

Twenty subjects have been studied in ages comprised between 48 and 68 years, distributed in three groups:

- Group A (n=5): healthy control subjects with normal ophthalmological exploration criteria in both eyes (BE) and without family history of glaucoma.
  - Best corrected visual acuity (BCVA) in the logMAR scale <+0.3.
  - Intra-ocular pressure (IOP) < 21 mm Hg.
  - Refractive error < 5 spherical equivalent dioptres or 3 astigmatism dioptres.
  - Normal Humphrey static perimetry (HVF).

- Group B (n=10): experimental patients recruited in the Neuro Ophthalmological Unit of the Príncipe de Asturias University Hospital, affected by severe bilateral optic neuropathy and with the following inclusion criteria for both eyes:
  - BCVA ≤ +1.00.
  - Mean deviation (MD) in HVF < -12 dB.
  - Reduction of the mean thickness of the retinal
nervous fiber layer (RNFL) On 360º under 1% (Avg Thickness/OCT).

Normal external retinal function in mf ERG.
• Group C (n=5): blind control subjects, referred by the National Organization of Spanish Blind (ONCE) that fulfill the following criteria for both eyes:
  No conscious perception of light (LP).
  Annulled pupil reflexes to light.
  Ophthalmoscopically diagnosed optical atrophy.

With the exception of a history of insomnia in a blind patient, none of the subjects included in the study exhibited a systemic or psychiatric disease or was under medical treatment. In none of the cases did they work during the night. All the participants signed an informed consent according to the Helsinki Declaration. The study was approved by the Ethical Committee of the Príncipe de Asturias University Hospital.

Methods

All the control subjects and experimental patients had a routine ophthalmological exploration including BCVA measurement, refraction, pupil reflexes, slit lamp biomicroscopy, IOP measurement, ocular fundus study and retinographies. In group A and B subjects a number of supplementary explorations were carried out, comprising HVF, OCT assessment and mf ERG tests in order to determine and document the severity of the optic neuropathy. The values obtained in each eye were averaged. In all cases melatonin concentration in the saliva was determined by means of RIA.

The BCVA measurement was made with a Logarithmic Visual Acuity optotype (LogMAR) Chart 2000 «New ETDRS» from Precision Vision. The visual field study was made with a Humphrey 740 computerized perimeter (Humphrey Instruments Inc.), utilizing the SITA 24-2 strategy with a Goldman stimulus III. In all cases the adequate correction was adapted for the exploration distance and the data corresponding to a second exploration were included. Only the visual field results with unacceptable reliability were included (under 25% of fixation losses, false positives and false negatives).

Optic Coherence Tomography (OCT)

The explorations were made utilizing OCT Stratus TM 4.0.2 version 0052 (Carl Zeiss Meditec, Inc, Dublin, California) by a single experienced operator (JPM). For studying the peri-papillary RNFL thickness, the Fast RNFL Thickness 3,4 protocol was utilized. This protocol carries out three circular scans with a diameter of 3.4 mm in 1.92 seconds and compresses them in a single tomography. Each scan comprises 256 A-scans. All the measurements were made under the same environmental lighting conditions and under pupillary dilatation. In all cases internal fixation was utilized (green target light inside the ocular globe). Three tomographies were obtained with an adequate single (above 5) for each eye which were recorded for calculating the mean values. In each eye the mean RNFL thickness measurement was studied at 360º Avg Thickness, automatically calculated by the OCT software.

Multifocal electroretinography (mf/ERG)

To obtain the mf/ERG values a VERIS multifocal electrophysiology system was utilized (EDI, San Mateo, USA). In all cases the pupils were dilated with tropicamide and phenylephrine 2.5% eye drops, placing the adequate correction in front of the eye for the exploration distance. The patients were given 15 minutes to adapt to the exploration lighting conditions (6 cd/m²) and the contralateral eye was occluded. A bipolar Burian-Allen, contact lens-type electrode protected with methylcellulose was placed over the cornea with previous topical anesthesia, with another ground electrode in the right ear lobe.

The stimulation utilized comprised 103 hexagonal elements aligned in concentric circles covering a visual angle of 20-30º at both sides of the fixation point. The stimulations were displayed on a TV screen of a SONY Trinitron MultiSCAN model E500 with a frequency of 75 Hz and a maximum luminance of 100-200 cd/m² (white) and minimum luminance of <1 cd/m² (black), following a pseudorandomized m-binary sequence. The contrast between the white and black stimulation was of 90% or greater and at all times the patient fixation was monitored. The signals were amplified 100,000 times and a screening was established with a range between 10 and 300 Hz. The total duration of the recording was of eight minutes. The recordings were analyzed by means of proprietary software developed in MatLab.
Determination of melatonin in saliva

The concentration of melatonin in saliva was determined by means of RIA (Melatonin direct RIA saliva, RE29371, IBL Immuno Biological Laboratories, Germany). This test has an analytical sensitivity of <0.086 pg/ml, an intra-essay variability of 2.7-8.1% and inter-essay variability of 12.6-14.6%.

Six samples of saliva were collected for each subject between 10 p.m. and 3 a.m. in darkness after midnight. The last sample was obtained after direct exposure to a brilliant 600 lux white light during one hour. The samples were frozen at -20º C. At this temperature, melatonin remained stable in saliva for one month (10). The determinations of melatonin were made in duplicate for each sample and the samples of the same model were measured in the same assay. The melatonin nocturnal suppression test (MNST) induced by exposure to light is considered positive with a 30% reduction in the concentration of melatonin compared to the previous level (11).

Statistical analysis of the data

The data were expressed in mean values and standard deviation (SD). The differences between control subjects and patients were statistically assessed with the t for student test. The statistical study was made with SPSS 14.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

The demographic data of the 20 subjects included in the study are summarized in table 1. No statistically significant differences were found as regards age between the 3 groups of subjects (p=0.5).

The data obtained from the ophthalmological exploration of the group A subjects (n=5) were normal in all cases. The biomicroscopic study, IOP measurement (14.56 SD 1.76 mm Hg) and ocular fundus exploration were normal.

Table 2 describe the clinical evaluation data of each patient in group B (n=10). The neuro ophthalmological assessment revealed several optic neuropathy causes: Five patients exhibited open angle primary glaucoma (OAPG), 3 patients had Anterior Ischemic Optic Neuropathy (AION) and 2 exhibited Kjer-type hereditary optic atrophy.

Table 3 illustrates the mean values of the variables analyzed in the ophthalmological exploration

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**Table I. Demographic Data**

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 5)</th>
<th>Group B (n = 10)</th>
<th>Group C (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AGE (years)</strong></td>
<td>Mean SD</td>
<td>Range</td>
<td>Mean SD</td>
</tr>
<tr>
<td></td>
<td>60.20 SD 3.94</td>
<td>58-63</td>
<td>61.30 SD 5.47</td>
</tr>
<tr>
<td><strong>GENDER</strong></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>2 (40%)</td>
<td>3 (60%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td><strong>RACE</strong></td>
<td>Caucasian</td>
<td>Colored</td>
<td>Caucasian</td>
</tr>
<tr>
<td></td>
<td>5 (100%)</td>
<td>5 (100%)</td>
<td>9 (90%)</td>
</tr>
</tbody>
</table>

SD = Standard Deviation.

**Table II. Group B. Clinical Assessment**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Diagnostic</th>
<th>BCVA (LogMAR)</th>
<th>HVF/DM (dB)</th>
<th>OCT (µm) Avg Thick</th>
<th>mERG</th>
<th>MNST</th>
</tr>
</thead>
<tbody>
<tr>
<td># 1</td>
<td>OAPG</td>
<td>0.16</td>
<td>-19.55</td>
<td>47</td>
<td>Normal</td>
<td>Negative</td>
</tr>
<tr>
<td># 2</td>
<td>OAPG</td>
<td>0.25</td>
<td>-19.36</td>
<td>44.79</td>
<td>Normal</td>
<td>Negative</td>
</tr>
<tr>
<td># 3</td>
<td>OAPG</td>
<td>0.13</td>
<td>-13.75</td>
<td>54.07</td>
<td>Normal</td>
<td>Negative</td>
</tr>
<tr>
<td># 4</td>
<td>AION</td>
<td>0.85</td>
<td>-22.86</td>
<td>42.81</td>
<td>Normal</td>
<td>Positive</td>
</tr>
<tr>
<td># 5</td>
<td>Kjer</td>
<td>1.00</td>
<td>-23.98</td>
<td>59.63</td>
<td>Normal</td>
<td>Positive</td>
</tr>
<tr>
<td># 6</td>
<td>OAPG</td>
<td>0.16</td>
<td>-17.81</td>
<td>53.45</td>
<td>Normal</td>
<td>Negative</td>
</tr>
<tr>
<td># 7</td>
<td>AION</td>
<td>0.25</td>
<td>-20.95</td>
<td>46.75</td>
<td>Normal</td>
<td>Positive</td>
</tr>
<tr>
<td># 8</td>
<td>AION</td>
<td>0.64</td>
<td>-16.57</td>
<td>55.25</td>
<td>Normal</td>
<td>Positive</td>
</tr>
<tr>
<td># 9</td>
<td>Kjer</td>
<td>0.87</td>
<td>-23.84</td>
<td>49.37</td>
<td>Normal</td>
<td>Positive</td>
</tr>
<tr>
<td># 10</td>
<td>OAPG</td>
<td>0.30</td>
<td>-26.87</td>
<td>45.40</td>
<td>Normal</td>
<td>Negative</td>
</tr>
</tbody>
</table>

OAPG: open angle primary glaucoma; AION: Anterior ischemic optic neuropathy; BCVA: Best corrected visual acuity; HVF: Humphrey visual field; SD: Standard deviation; OCT: Optical Coherence Tomography; Avg Thick: average thickness; mERG: multifocal electoretinogram; MNST: Melatonin nocturnal suppression test.
of the Group A and B subjects. The differences between both groups were statistically significant in what concerns BCVA LogMAR, MD in HVF and Avg Thickness in the OCT exploration (P<0.001). The mf ERG tests showed normal function of photoreceptors both in the 3-D record and the waveform map (fig. 1), in all the subjects of Group A and B.

The subject of Group C (n=5) did not exhibit LP awareness in BE and their demeanor and gestures were those of blind people. Pupil reflexes to the light were annulled and the ocular fundus exploration revealed optic atrophy in both eyes with the exception of patient #1 who was unable to undergo said explorations due to bilateral phthisis bulbi (Table 4). The data obtained from the ophthalmological explorations of these subjects were not included in the statistical analysis. Their participation as negative controls was limited to the determination of melatonin in saliva.

**Melatonin Nocturnal Suppression Test (MNST)**

In Group A, MNST was positive in 100% of cases. In Group B, five of the patients had negative MNST results (50% of cases) and a further five patients had positive results (50% of cases) (table 2). In Group C, MNST was negative in 100% of cases.

**DISCUSSION**

In our series, 100% of blind subjects of Group C, in the absence of conscious LP and with annulled

<table>
<thead>
<tr>
<th>MAVC LogMAR Minutes arc</th>
<th>HVF/DM (dB)</th>
<th>OCT (µm) Avg Thick</th>
<th>mfERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Subjects</td>
<td>0.09 SD 0.51</td>
<td>0.51 SD 0.51</td>
<td>113.29 SD 3.94</td>
</tr>
<tr>
<td>Experimental subjects</td>
<td>0.46 SD 0.34</td>
<td>-20.55 SD 3.94</td>
<td>49.85 SD 5.46</td>
</tr>
</tbody>
</table>

Table III. Ophthalmological Exploration. Statistical Analysis of Data (Groups A and B)

- BCVA: best corrected visual acuity; HVF: Humphrey visual field; MD: mean deviation; OCT: Optical Coherence Tomography; Avg Thick: average thickness; mfERG: multifocal electroretinogram; MNST: Melatonin nocturnal suppression test.

![Field View](image1)

**Fig. 1:** mfERG. The 3-D illustration and the waveform map show normal function of the external retina.
pupil reflexes exhibited negative MNST results. However the MNST results in group B patients, affected by severe optic neuropathies in BE and with variable degrees of visual acuity, were more heterogeneous as the MNST test was negative only in 50% of cases.

The MNST light exposure tests could be very useful to determine the functional integrity of the RHT which connects the retina with the pineal gland through the SCN (12). In this regard it has been demonstrated that in the absence of conscious light perception some blind people exhibit nocturnal melatonin suppression (11) and circadian rhythms causing the segregation of melatonin in 24 hour cycles (13,14). This would indicate that light acts through the SCN. Similar data obtained in animal experimentations with rats support these results (15).

The fact that blind people maintain nonvisual responses to light (11,13,14) supports the hypothesis that the eyes of mammals include at least two anatomically and functionally different systems that detect light: 1) on the one hand, the ordinary visual system which forms images and involves rods and cones and, 2) on the other hand a photoreceptive system which detects light but does not form images and synchronizes the circadian rhythms. The RHT, which originates in a sub-population of light-sensitive retinal ganglionary cells, the ip RGC, projects to the SCN and forms part of the system (16).

The discovery of a new opsine, melanopsin, in the eye of mammals represented a considerable development in the knowledge of the way in which light stimulates nonvisual functions (17). Surprisingly, melanopsin is not located in photoreceptors with the rhodopsine of the rods and the opsines of the cones, but in the ip RGC (18).

As the effect of light on the suppression of melatonin is dosage dependent (19), it could be thought that the severity of visual loss and the type of disease could have an influence on this effect. In clinical studies made with patients having dysfunctions in the cone system or pigmentary retinosis, it has been demonstrated that the visual loss has a low impact on the circadian system (20,13). In our series, all the patients exhibited severe optic neuropathies in both eyes with the normal function of their external retina as proved with mf ERG but, regardless of the intense loss of retina ganglionary cells, 50% of these patients exhibited a positive MNST, possibly due to a greater resistance of the ip RGC to the lesions caused by these diseases (21). In addition, we observed that 100% of patients with OAPG exhibited a negative MNST, which suggests that in our series the ip RGC are more sensitive to glaucomatous damage.

On the basis of the above preliminary results, we can conclude that 50% of patients with severe optic neuropathies exhibit alterations in the non-visual responses to light in terms of melatonin nocturnal secretion suppression, which would involve a dysfunction of the ip RGC in spite of the functional normality of photoreceptors. Additional studies (e.g., multifocal evoked potentials) could provide more information about the degree of functionality of the retina ganglionary cells in these patients in order to predict their possible association with circadian rhythm disorders.

**REFERENCES**


