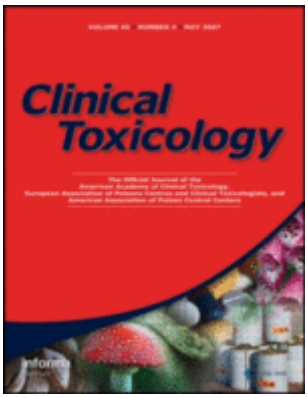

Retinal and Visual Pathway Alterations After Severe Acute Occupational Elemental Mercury Poisoning. Report of 29 cases.

Journal:	*Clinical Toxicology*
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Abstract:	Objective: To report visual findings in a group of workers exposed to very high concentrations of mercury in a factory in northern Spain at the end of 2012. Methods: Twenty-nine patients exposed to mercury vapor and 11 healthy controls were evaluated. Blood and urine samples were collected to assess mercury levels. Fifteen workers underwent late chelation for heavy metal intoxication. Complete visual examination, including optical coherence tomography (OCT), visual field test (VFt), contrast sensitivity (CS) and color discrimination were assessed. Retinal function was evaluated using the ISCEV protocol for full-field electroretinography (ffERG), pattern electroretinography (PERG), and multifocal electroretinography (mfERG). In addition, systemic symptoms, possible erethism, and electromyography (EMG) were recorded. Values were compared between non-chelated and late chelated patients. Results: Visual acuity (VA) was slightly affected. Loss of CS in all frequencies and color vision alterations were found. VF alterations were registered in 62.1% of patients. OCT scans showed no morphological changes. All patients presented latencies > 100ms and reduced amplitudes of P100. The ffERG and PERG showed changes, suggesting that both the outer and inner retina layers were involved. mfERG indicated that central retinal function was also significantly depressed. Twenty-six workers had symptoms of erethism. The EMG showed signs of mixed sensorimotor polyneuropathy and multiple mononeuropathy alterations. No differences were found between groups. A significant negative correlation between blood mercury levels, VA and ffERG was observed. Conclusion: Advanced visual functions were significantly impaired independently of mercury levels. Delayed chelation had no added benefit. Although neurological and visual pathway involvement was clearly demonstrated, there were also evidences of a direct functional

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Keywords: Mercury poisoning; Mercury vapor; Occupational exposure; Retinal toxic effects; Optical coherence tomography, Full-field electroretinography, pattern electroretinography, multifocal electroretinography.

Introduction

Acute or subacute occupational elemental mercury poisoning is uncommon, but may have important consequences on the visual pathway, due to the neurotoxicity of mercury [1,2]. However, the magnitude of the possible damage on the retinal structures is still unclear. Experimental studies have shown the presence of mercury in the retina and choroid [3], but others have limited its presence to the retinal pigment epithelium and external layers of the neuroretina [4,5].

Electrophysiology tests and current clinical examination techniques such as autofluorescence or optical coherence tomography (OCT) suggest that, besides central nervous system (CNS) poisoning, there is also retinal involvement and that not all visual functional alterations are caused by high visual pathway damage [6,7,8].

Physiological and morphological retinal changes due to mercury toxicity have been demonstrated in animal models; but there are few reports on human retinal involvement due to occupational poisoning: the last large series of patients intoxicated by mercury was reported before the latest retinal diagnostic techniques, such as spectral domain OCT (SD-OCT) and mfERG became clinically available. There is only one report of OCT examination in patients affected to chronic mercury exposure [7,8], and only one that includes mfERG in these patients [6]. To our knowledge, this is the first study to use SD-OCT, mfERG and pattern electroretinography (PERG) evaluations in a series of patients inadvertently acutely exposed to high levels of elemental mercury vapor.

We report visual pathway alterations in 29 workers exposed to very high levels of mercury for fourteen consecutive days during maintenance work in a factory

in Northern Spain at the end of 2012. This was one the most severe acute elemental mercury intoxications in the European Union ever reported.

Material and Methods

Patients

Forty-nine workers, according to official sources, were inadvertently exposed to elemental mercury vapor during maintenance work in a heat exchanger. The incident occurred between November 19th and December 2nd, 2012, in a metal manufacturing plant in Northern Spain. According to the workers' story, when they entered the workspace, they found many balls of mercury spread over the floor, which were not removed. Some days after finishing their work, many of them reported physical complaints, including asthenia, headache, lumbago, cough, bitter taste, dental pain, gum inflammation and bleeding, epigastric and abdominal pain, among other symptoms, which were initially attributed to a viral infection.

After the initial symptomatology, most patients presented with erethism, ~~with~~ fatigue, irritability, aggressiveness, anxiety, depression and insomnia. In addition, they presented neurological manifestations including tremor, peripheral polyneuropathy, weakness, headache, cognitive disorders, ~~and~~ dizziness, and digestive manifestations such as diarrhea and abdominal cramps. Also, many presented visual complaints such as blurred vision, ocular irritation, dry eye, burning or scratchy sensation, eye redness, and sensitivity to light.

Blood and urine mercury levels were measured from the second week of the exposure, and were above the recommended biological limits for occupational exposure [9,10], reaching 500-900 µg/L in blood and 600-1830 µg/g Cr in urine. Before the occupational exposure, urine mercury levels were measured some of the workers, showing levels of < 3µg/g Cr. However, there was no quantitative reference data on the level of mercury exposure at the time of the event.

Despite the range of early symptoms, only three workers received early chelation with dimercaprol (BAL), which was prematurely interrupted due to severe adverse reactions to this compound.

Between September 2013 and the end of 2014, 44 of the 49 officially affected patients approached the Clinical Toxicology Unit, Medical Science Institute (ICIME), University of Valladolid (Spain) for an independent assessment.

After evaluating each case, ancillary tests and actions were proposed based on clinical data. Late chelation (8-12 months after the exposure with oral 2,3-dimercapto-1-propanesulfonic acid (DMPS) for a minimum of one week, was proposed, according to severity criteria in local hospitals, and was administered in 15 patients. Twenty-nine patients who presented visual symptoms at the second appraisal were referred for complete visual evaluation at the IOBA-Eye Institute, University of Valladolid.

Ophthalmic examination

In the initial examination, previous ocular, neural or systemic disease that might affect or interfere with visual examinations was ruled out.
Informed consent was obtained from all patients before the ophthalmic assessment. The procedures complied with the tenets of the Declaration of Helsinki and were approved by the Ethics Committees of the Clinic University Hospital and IOBA-University of Valladolid, Spain.

Twenty-nine patients underwent a full ophthalmic examination including best corrected visual acuity (BCVA) on the ETDRS scale; slit-lamp examination; intraocular pressure and funduscopy and OCT with specific evaluation of central retinal thickness (CRT) (3D-OCT 2000, Topcon Inc., Tokyo, Japan) and examination of the retinal nerve fiber layer thickness (RNFLT) (OCT Stratus 3000 Zeiss Meditec, Oberkochen, Germany). Color vision was examined by the Farnsworth-Munsell (FM) 28 Hue (Luneau Ophtalmologie, Paris, France) and contrast sensitivity (CS) by the CSV-1000 chart (Vectorvision, Greenville, USA). The FM 28 Hue results were scored in two ways. First, a color confusion index (CCI) was calculated for each participant for the statistical analysis [11,12].

Secondly, a clinical diagnosis of the type of loss was made by plotting the response on a standard score sheet, allowing determination of the axis of color confusion.

Visual field (VF) was assessed using a Humphrey 750i visual field analyzer (Carl Zeiss, Oberkochen, Germany) and the central 30-2 SITA fast strategy protocol. Only tests that met the criteria (low (<20%), false positive, false negative and fixation loss parameters) were evaluated.

Retinal function was evaluated by full-field electroretinography (ffERG), pattern electroretinography (PERG) and multifocal electroretinography (mfERG), using the International Society for Clinical Electrophysiology in Vision (ISCEV) protocol [13]. ERGs were recorded using a ganzfeld stimulator (Metrovision, Lille, France) with a corneal electrode according to the international ISCEV protocol [14]. mfERGs of both eyes were made with scaled hexagons stimulating 61 zones. Four patterns of abnormal mfERG amplitude responses were assessed: paracentral loss, foveal loss, peripheral loss and generalized loss, as described by Maturi *et al* [15].

Additional tests

Electromyography (EMG): nerve conductance was assessed to evaluate peripheral neuropathy using a standard protocol (Nihon Kodhen, Model MEB-9400, Irvine USA). Sensory and motor nerve conduction velocities were determined in the median and peroneal nerves. Amplitude (μ V), latency (m/s) and conductance (m/s) were evaluated.

Statistical analysis

The BCVA was recorded on an ETDRS scale and converted to the logarithm of the minimal angle of resolution (LogMAR) for statistical analysis. All visual acuity results are expressed in LogMAR units with the Snellen equivalent in parentheses. Categorical variables were analyzed using Fisher´s exact test or the chi-square test. The t-test was used to compare mean values between parametric values. Pearson's correlation test was used to evaluate the correlation between ophthalmic findings and blood and urine mercury levels. For non-normally distributed data, continuous variables were analyzed using the

Wilcoxon rank-sum test. For repeated measures, the Wilcoxon signed-rank test was used. Spearman's test was used to correlate non-normally distributed data. For all tests a *p*-value <0.05 was considered to be significant.

For the statistical analysis, participants were divided into two groups: group 1(G1) (n=14) consisted of patients who did not receive late chelation and group 2 (G2) (n=15) of those who received late chelation. For the ffERG tests, an age-matched control group (n=11) was included in the between-group comparisons. The statistical analysis was made using the SPSS 17.0 statistical program (SPSS Inc. Chicago, IL).

Results

All 29 patients were male with a mean age of 40.62 ± 8.04 years (range 25-56). The mean urinary mercury level at early stage was 302.86 ± 405.35 μg/g Cr (range 10-1830) and the mean blood mercury level was 392.93 ± 273.84 μg/L (range 26-961). There were significantly increased blood and urine mercury levels in samples obtained from G2 compared with G1. The main clinical baseline characteristics and EMG results are summarized in table 1.

Ophthalmological findings

The main ophthalmic findings are summarized in table 2.
Decreased visual acuity (< 20/20) was detected in nine (64.3%) patients in G1 and five (33.3%) patients in G2, without significant differences. Fifteen patients (51.7%) reported additional ocular complaints.

The VA, color vision, CS, VF and OCT results are shown in table 2. Acquired dyschromatopsia, especially in the blue-yellow range on FM 28 Hue, was found in thirteen (44.8%) patients. The mean ICC was 1.642 ± 1.183. No significant between-group difference in the ICC was found (Table 2).
Twenty-eight (96.5%) patients presented alterations in achromatic CS in ≥ 1 of the four spatial frequencies, especially in the high frequencies, without significant between-group differences (Table 2 and S1).

Twenty-one (72.4%) patients had visual field test alterations. The most prevalent patterns were concentric constriction (17 eyes, 29.3%), scattered defects (6 eyes,10.3%), hemi-field defects respecting horizontal and vertical meridians (5 eyes, 8.62%), nasal defects (4 eyes, 6.89%) and arcuate defects (2 eyes, 3.44%). There were no significant between-group differences in patterns, MD or VFI (Table 2). No significant between-group differences were found in the mean value of CRT [16] or RNFLT [17] (Table 2).

A significant negative correlation between blood mercury levels and BCVA was observed in the correlation analysis between blood mercury levels and all these variables (Table 3).

Electrophysiology function assessment

ffERGs

ffERG was recorded in 28 patients in both eyes and in 11 age-matched controls. The amplitude of the *a*- and *b*-wave for the scotopic rod response (SRR) of ERG was significantly higher in patients than in controls (Table 4 and S2).

A significant negative correlation between blood mercury levels and ERG amplitudes of the *b-wave* in SRR, maximal scotopic response (MSR), sum of oscillatory potential (OP) and 30Hz Flicker responses, was found in G1. The same correlation with the *b-wave* amplitudes in SRR and the sum of oscillatory potentials was found in G1; and with the 30Hz Flicker amplitudes and latency in the whole group of patients (Table 5).

PERG

PERG was performed in 27 patients for the RE and 26 patients for the LE. (Table 6 and S3). The amplitude of P50 and N95 was significantly diminished in patients compared with reference values [18] in both eyes. There were no significant between-group differences in implicit times of the P50 and N95 components (Table 6 and S3). There was no correlation between PERG values and blood mercury levels.

PVEP

PVEP was recorded in 29 patients. The mean implicit P100 times were significantly higher and the amplitudes lower than the reference values for both 60 and 30-minute checks stimuli [18,19]. There were no significant between-group differences (Table 6 and S3). There was no correlation between VEP values and blood mercury levels.

mfERG

mfERG was recorded in 26 patients. The most prevalent patterns were peripheral loss (16 eyes, 30.7%), central loss (7 eyes, 13.4%), and paracentral defects (5 eyes, 9.6 %). Conversely, no depression of the amplitude responses to full field ERG was observed in 22 eyes (42.3%). As the peripheral pattern was the most frequent, P1 amplitude in the peripheral rings of the mfERG was analyzed, finding a significantly lower value in patients in rings 5-10º and >15º compared with reference values [15,20]. There were no between-group differences in mfERG patterns (Table7 and S4) and no correlation between mfERG values at rings 5-10º, 5-10º, 10-15º and >15º and blood mercury levels.

In addition, when mfERG was compared with the visual field, fourteen (48.3%) patients showed lower local agreement in the location between the mfERG defects and the total deviation of visual sensitivities of < 5% recorded in the visual field tests. There were no between-group differences.

Additional tests

Electromyography

EMG was performed in 27 patients and the results showed different types of patterns of abnormalities and decreased nerve conduction velocity in most (Table1). There were no significant between-group differences. There was no correlation between blood mercury levels, nerve conduction velocity and the P100 component in PVEP.

Discussion

Mercury vapor is a significant source of mercury load in occupational exposures as it is odorless, colorless and tends to accumulate in poorly-ventilated areas.

Once the lungs have absorbed inhaled vapor, it may reach different tissues via the bloodstream, with the primary targets being the CNS and eyes [2,21]. When oxidized, it cannot penetrate the blood-barrier again, remaining in the tissues for prolonged periods [2,7,8,21].

Neurological and visual pathway involvement due to mercury toxicity has been widely described [2,21,22]. The effects of long-term exposure include symptoms that range from tremor, neuropathy, changes in personality known as erethism, speech disturbances, delirium or rigidity to symptoms of visual field defects, reduced visual acuity, color vision and night vision, or decreased contrast sensitivity, [2,21,23,24]. However, the introduction of electrophysiology tests has established that there is also retinal involvement and that not all alterations of the visual alterations are due to CNS and visual pathway involvement [6].

The initial complaints of these patients were initially attributed to a viral infection, delaying the diagnosis of mercury poisoning. At diagnosis, mean urine (mean: 302,86 μ g/g Cr) and blood (mean: 392.93 μ g/L) mercury levels significantly exceeded the maximum accepted level for occupational exposure (<30 μ g/g Cr and 10 μ g/L respectively) [9,10]. In such cases, the mainstay of treatment is chelation; however early chelation was only performed in three patients and was prematurely interrupted due to severe adverse reactions. Fifteen workers received delayed chelation, which did not result in satisfactory relief of any symptom.

Twenty-six workers presented symptoms of erethism. Some also presented typical cognitive symptoms of mercury poisoning, such as disturbances in memory and attention [21,22]. Tremor in the hands, head and eyelids, a late symptom of mercury poisoning, was also seen. The EMG showed signs of mixed sensorimotor polyneuropathy and multiple mononeuropathy alterations 12-18 months after the exposure.

Visual acuity was minimally affected, as only nine patients from G1 and five from G2 showed a reduction; however advanced visual functions were

significantly impaired, apparently independently of mercury levels since there was only a significant negative correlation between BML, BCVA and ffERG. Color vision and CS impairment at high spatial frequencies were found, with the most frequently observed being color vision alteration in the blue-yellow range. These findings agree with previous studies [23,25-27].

The most prevalent pattern in the visual field tests was concentric constriction of the visual field (17 eyes, 29.3%), also in agreement with previous studies [28,29]. This visual impairment may have a central origin, as it has been explained by lesions in the calcarine cortex [31]. The increase in the implicit time of P100 means a delay in conduction and involvement of the visual pathway. This finding was also reported by da Costa et al in 2008 [18]. However, in the current series there was also significant functional retinal involvement, since the same patients showed retinal dysfunction in the ffERG, PERG and mfERG tests, revealing loss of generalized retinal responses and in the central retinal areas.

The ffERG showed changes in the scotopic rod response and the oscillatory potentials of the ISCEV protocol, suggesting that the rod cells were impaired by acute mercury-vapor intoxication. Although we found no differences in the MSR, 30Hz Flicker and SFCR tests compared with controls, the amplitude of P50, which is critical in assessing macular cone function, showed a significant decrease, suggesting that the cone and ganglion macular cells were also impaired. This reinforces the idea that both the outer and inner retina layers were involved in these patients. Finally, the mfERG results were further evidence of damage to the photoreceptor pathway in mercury poisoning, since the response amplitudes showed a loss of the retinal response within the 50 central degrees explored, as found in other studies [6].

The latency and amplitude of TPEV showed no correlation with BML; however all patients presented latencies > 100ms and reduced amplitudes of P100. Although these results typically occur in optic neuropathies and visual cortex abnormalities, they may also be associated with macular disease, especially when interpreted in conjunction with retinal function tests (PERG and ffERG).

Our results were in agreement with those found by da Costa et al in patients with mercury poisoning [18].

Despite the functional retinal involvement and in contrast to the results obtained by Ekinci et al [7,8], OCT examination did not reveal structural changes on RNFL, macular (CRT) and choroid thickness when the results were compared with normalized reference values [16,17]. These differences might be related to the intensity and the form of the intoxication, as our patients were exposed to higher levels of mercury in a short time compared with the long exposure times of the workers examined by Ekinci et al [7,8].

Limitations

The study had some limitations. First, despite the initial assessments performed prior to the accident, there were no environmental measurements of mercury during the occupational incident.

Secondly, it is probable that only the most-severely affected patients were evaluated by the IOBA. The time between the exposure and the IOBA assessment and the fact that patients lived far away from the IOBA was not conducive to a correct follow up.

Thirdly, with respect to the electrophysiological tests, only ffERG values were compared with controls. Therefore, the results of the remaining comparisons should be considered with caution, since reference values were gathered from the literature.

Finally, OCT technology has evolved so rapidly in recent years that, with current OCT based on swept-source or ultra-high resolution, it might have been possible to detect changes in the retinal or choroid structures.

Conclusions

VA was slightly affected, and VF defects were more frequent after severe acute occupational elemental mercury poisoning. The most prevalent VF alteration was peripheral reduction of the visual fields, but central involvement was also found. These defects could be either of retinal and/or neurological origin (visual pathway), considering the mfERG results.

Visual alterations seemed to be independent of mercury levels. No structural anatomical retinal changes were found by SD-OCT, but the new OCT systems might allow the structural bases of these alterations to be established. Delayed chelation added no further benefits.

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Table 1. Baseline characteristics, laboratory and electromyography findings.

		Group 1	Group 2	p-value	Total patients
N		14 (40.3%)	15 (51.7%)	-	29
Age		40.93 ± 7.76	40.33 ± 8.57	0.8464	40.62 ± 8.05
Smoking		8 (57.1%)	10 (66.7)	0.8845	18 (62.1%)
Hypertension		1 (7.1%)	1 (6.7%)	0.4694	2 (6.9%)
Dyslipidemia		2 (14.3%)	2 (13.3%)	0.1288	4 (13.8%)
Hg Blood (µg/L)		274.86 ± 201.8	503.13 ± 291.92	**0.0219**	392.93 ± 273.85
Hg Urine (µg/g Cr)		99.21± 97.27	492.93 ± 489.56	**0.0014**	302.86 ± 405.36
Erethism		11 (78.6%)	15 (100%)	0.0996	26 (89.7%)
EMG patterns	N	1(7.1%)	-	0.4828	1 (3.4%)
	SP	8 (57.1%)	6 (40%)	0.4661	14 (48.3%)
	ASP	1 (7.1%)	6 (40%)	0.0801	7 (24.1%)
	MM	2 (14.3)	2 (13.3%)	1.0	4 (13.8%)
	N/A	2 (14.3%)	1 (6.7%)	0.5977	3 (10.3%)
EMG CVA	SM (ms)	30.63 ± 3.82	35.33 ± 8.26	0.6026	33.5 ± 7.13
	MN (ms)	36.29 ± 6.19	40.24 ± 6.73	0.1123	38.78 ± 6.65
Psychiatric treatment		3 (21.4%)	10 (66.6%)	**0.0253**	13 (44.8%)

Group 1: Patients without chelation; Group 2. Chelated patients; EMG: Electromyography; N: normal pattern; SP: sensorimotor polyneuropathy; ASP: axonal sensory polyneuropathy; MM: multiple mononeuropathy; N/A: not performed. EMG CV: Electromyography: conduction velocity assessment; SN: Sensory nerve; MN: Motor nerve.
Reference values [32,33]: normal velocity conduction in SN >40ms. Normal speed conduction in MN >49ms.

Table 2. Ophthalmic examination findings. BCVA; CV; CCI; CS; VFt; and OCT

		Group 1		Group 2		p-value		Total patients		Reference values
N (eyes)		28		30		-		58		-
BCVA Logmar [Snellen]		0.017 ± 0.151 [0.887 ± 0.238]		0.078 ± 0.865 [0.950 ± 0.172]		0.631		0.048 ± 0.126 [0.920 ± 0.205]		0.0 [6/6]
CVS		7 (50%)		6 (40%)		0.715		13 (44.8%)		-
CCI		1.872 ± 1.407		1.278 ± 0.597		0.146		1.642 ± 1.183		1.0
CSA	Eye	RE	LE	RE	LE	RE	LE	RE	LE	-
	CS3	9 (64.29%)	10 (71.43%)	7 (46.67%)	11 (73.33%)	0.4621	0.9999	16 (55.17%)	21 (72.41%)	-
	CS6	12 (85.71%)	10 (71.43%)	10 (66.67%)	10 (66.67%)	0.3898	0.9999	22 (75.86%)	20 (68.97%)	-
	CS12	12 (85.71%)	11 (78.57%)	11 (73.33%)	11 (73.33%)	0.6513	0.9999	23 (79.31%)	22 (75.86%)	-
	CS18	13 (92.86%)	11 (78.57%)	13 (86.67%)	13 (86.67%)	0.9999	0.6513	26 (89.65%)	24 (82.75%)	-
VFt	Eye	RE	LE	RE	LE	RE	LE	RE	LE	-
	MD	-5.52 ± 7.33	-6.88 ± 8.18	-5.76 ± 8.7	-6.86 ± 9.11	0.8614	0.8272	-5.64 ± 7.92	-6.87 ± 8.52	0.0
	VFI	88.0 ± 18.4	85 ± 20.3	85.4 ± 24.1	85.7 ± 23.0	0.7093	0.4294	86.7 ± 21.2	85.4 ± 21.4	100%
	Total	8 (57.1%)		10 (66.6%)		0.7104		18 (62.1%)		-
OCT	Eye	RE	LE	RE	LE	RE	LE	RE	LE	-
	CRT	249.8 ± 15.9	248.9 ± 18,8	249.0 ± 25.5	247.4 ± 23,5	0.9288	0.8537	249.4 ± 21,0	248.1 ± 20,7	233.6±19.7
	RNFLT	103.2 ± 13.2	100.4 ± 12.0	101.3 ± 7.77	100.1 ± 11.1	0.3536	0.3536	102.2 ± 10.5	100.2 ±11.3	100 ± 18

Group 1: Patients without chelation. Group 2. Chelated patients. EMG: Electromyography; CVS: Color vision scores; CCI: Color confusion index; CSA: alterations in the achromatic contrast sensitivity; CS3: spatial frequency at 3cycles/degree; CS6: spatial frequency at 6 cycles/degree; CS12: spatial frequency at 12 cycles/degree; CS18: spatial frequency at 18 cycles/degree; VFt: visual field test; DM: Mean deviation; VFI: Visual field index; OCT: optical coherence tomography; CRT: central retinal thickness; RNFLT: retinal nerve fiber layer thickness;

Table.3. Correlation between BML and BCVA

BML		Group 1	Group 2	Total patients
BCVA	RE	r=-0.56	r=-0.34	r=-0.36
		p=0.038	p=0.204	**p=0.049**
	LE	r=-0.54	r=-0.32	r=-0.37
		p=0.042	p=0.245	**p=0.042**
	Global	r=-0.54	r=-0.30	r=-0.36
		p=0.042	p=0.274	**p=0.048**

Group 1: Patients without chelation. Group 2. Chelated patients. BML: Blood mercury levels; BCVA: best corrected visual acuity.

Table 4. Full-field ERGs. Amplitude of a- and b-waves for the SRR, MSR, OP, Flicker 30Hz and SFCR

Full-field ERGs			Group 1	Group 2	p-values	Total patients	Control group (n=11)	p-values	95%CI
SRR	a-wave (μV)	RE	-15.62 ± 22.4	-13.98 ± 15.3	0.7819	-14.74 ± 18.6	-11.07 ± 53.48	0.745	
		LE	-7.76 ± 7.70	-8.21 ± 10.37	0.9015	-8.0 ± 9.06	-11.15 ± 60.88	0.783	
	b-wave (μV)	RE	270.21 ± 93.8	259.4 ± 60.8	0.7161	264.42 ± 76.3	177.55 ± 46.49	**0.0011**	[-136.8 to -36.9]
		LE	261.53 ± 90.0	262.46 ± 66.3	0.975	262.03 ± 76.7	172 ± 45.57	**0.0006**	[-142.2 to -42.5]
MSR	a-wave (μV)	RE	-161.46 ± 69.1	-159.13 ± 50.7	0.7296	-160.21± 58.8	-198 ± 50.49	0.065	
		LE	-166.86 ± 71.2	-152.88 ± 57.9	0.475	-159.37 ± 63.6	-155 ± 75.88	0.857	
	b-wave (μV)	RE	414.69 ± 106.4	414.66 ± 68.65	0.7821	414.67 ± 86.4	366.98 ± 138,9	0.115	
		LE	407.15± 102.6	401.13 ± 86.61	0.747	403.92 ± 92.6	381.48 ± 145.9	0.606	
OP	Amplitude (μV)	RE	564.66 ± 282.2	574.68 ± 239	0.9197	570.02 ± 254.9	206.24 ± 56.85	**0.0001**	[259.7-467.8]
		LE	519.66 ± 298.1	531.3 ±185.4	0.9002	525.93 ± 239.4	184.03 ± 54.08	**0.0001**	[248.69-453.91]
Flicker 30Hz	b-wave (μV)	RE	94.74 ± 39.0	86.64 ±15.1	0.4918	90.40 ± 28.5	82.81± 22.27	0.431	
		LE	98.33 ± 41.3	83.03 ± 22.4	0.2259	90.13 ± 32.8	82.6 ± 18.63	0.762	
SFCR	a-wave (μV)	RE	-14.51± 5.74	-12.05 ± 5.65	0.2642	-13.19 ± 5.72	-16.64 ± 2.57	0.062	
		LE	15.34 ± 5.55	-12.13 ± 6.45	0.1731	-13.62 ± 6.15	-18.08 ± 7.37	0.059	
	b-wave (μV)	RE	72.42 ± 26.24	58.74 ±17.4	0.2135	65.09 ± 22.6	64.43 ± 8.54	0.926	
		LE	72.69 ± 28.19	56.55 ± 19.2	0.0851	64.04 ± 24.7	57.65 ± 14.49	0.426	

SRR: scotopic rod response; MSR: maximal scotopic response; OP: oscillatory potential; Flicker 30Hz; SFCR: single flash cone response

Table 5. Correlation BML and b-wave amplitudes for the SRR, MSR, OP, Flicker 30Hz and SFCR

BML / Full-field ERGs		Eye	Group 1		Group 2		Total patients	
SRR	b-wave (μV)	RE	r=0.49	p=0.084	r=-0.35	p=0.197	r=-0.02	p=0.918
		LE	r=0.61	**p=0.025**	r=-0.12	p=0.648	r=0.18	p=0.353
MSR	b-wave (μV)	**RE**	r=0.61	**p=0.024**	r=-0.17	p=0.528	r=0.08	p=0.918
		LE	r=0.81	**p=0.001**	r=-0.02	p=0.935	r=0.26	p=0.171
Flicker 30 Hz	b-wave (μV)	**RE**	r=-0.06	p=0.825	r=-0.51	**p=0.047**	r=-0.24	p=0.214
		LE	r=0.06	p=0.841	r=0.08	p=0.772	r=-0.04	p=0.812
OP	Amplitude (μV)RE	**RE**	r=0.06	**p=0.023**	r=-0.57	**p=0.024**	r=-0.05	p=0.771
		LE	r=0.70	**p=0.007**	r=-0.47	p=0.073	r=0.10	p=0.607
SFCR	b-wave (μV)	RE	r=0.19	p=0.531	r=-0.31	p=0.259	r=-0.19	p=0.327
		LE	r=0.26	p=0.380	r=-0.07	p=0.787	r=0.07	p=0.708

Group 1: Patients without chelation. Group 2. Chelated patients. BML: Blood mercury levels; SRR: scotopic rod response; MSR: maximum scotopic response; OP: oscillatory potential; Flicker 30Hz; SFCR: single flash cone response

Table 6. PERG test (P50 and N95 components) and transient pattern visual evoked potential (tPVEP)

PERG			Group 1 Non-chelated	Group 2 Chelated	p-values	Total patients	Reference values	p-values	95% CI
P50	Amplitude (μV)	RE	4.2 ± 1.59	4.41 ± 1.5	0.9805	4.32 ± 1.51	7.6 ± 1.5	**0.0001**	[2.51-4.04]
		LE	4.56 ± 1.93	4.19 ± 1.72	0.6044	4.35 ± 1.78			[2.44-4.06]
N95	Amplitude (μV)	RE	-5.98 ± .548	-5.84 ± .314	0.4719	-5.90 ± .293	-1.7 ± 0.3	**0.0001**	[4.04-4.35]
		LE	-5.04 ± .688	-5.30 ± .637	0.1126	-5.19 ± .460			[3.30-3.67]

tPVEP			Group 1 Non-chelated	Group 2 Chelated	p-values	Total patients	Reference values	p-values	95% CI
P100-Da 60 R Lob	Amplitude (μV)	RE	9.72 ± 4.69	7.97 ± 3.94	0.2853	8.82 ± 4.33	14.16 ± 6.11	**0.0001**	[2.78-7.89]
		LE	8.82 ± 4.54	8.11 ± 4.32	0.6467	8.45 ± 4.36			[3.14-8.27]
	Latency (ms)	RE	116.78 ± 13.24	113.6 ± 6.39	0.4211	115.17±10.21	106.56 ± 4.62	**0.0001**	[-11.8 to -5.32]
		LE	119.4 ± 11.93	117.5 ± 7.51	0.5993	118.06 ± 9.71			[-14.6 to -8.33]
P100-Da 60 L Lob	Amplitude (μV)	RE	8.93 ± 4.07	8.83 ± 3.67	0.9476	8.88 ± 7.44	14.16 ± 6.11	**0.0009**	[2.23-8.33]
		LE	8.41 ± 3.36	7.61 ± 3.97	0.5608	8 ± 3.65		**0.0001**	[3.68-8.63]
	Latency (ms)	RE	114.64 ± 12.15	113.4 ± 5.73	0.8264	114 ± 9.24	106.56 ± 4.62	**0.0001**	[-10.5 to -4.37]
		LE	119.4 ± 12.31	117.5 ± 8.49	0.6173	118.47 ± 10.36			[-15.2 to -8.58]
P100-Da 30 R Lob	Amplitude (μV)	RE	8.1 ± 4.3	8.47 ± 3.23	0.7926	8.29 ± 3.72	16.54 ± 6.91	**0.0001**	[5.49-11.01]
		LE	7.65 ± 4.4	7.24 ± 3.53	0.7835	7.44 ± 3.91			[6.32-11.8]
	Latency (ms)	RE	116.57 ± 6.81	117 ± 5.83	0.8566	116.79 ± 6.21	112.12 ± 4.71	**0.0003**	[-7.11 to -2.22]
		LE	124.43 ± 18.42	120.47 ± 6.48	0.9474	122.38 ± 13.51		**0.0001**	[-14.3 to -6.15]
P100-Da 30 L Lob	Amplitude (μV)	RE	8.2 ± 4.56	7.71 ± 3.56	0.7471	7.94 ± 4.01	16.54 ± 6.91	**0.0001**	[5.81-11.39]
		LE	6.85 ± 4.74	6.14 ± 2.73	0.6222	6.48 ± 3.78			[7.29-12.82]
	Latency (ms)	RE	116 ± 8.03	118.2 ± 6.97	0.4367	117.14 ± 7.45	112.12 ± 4.71	**0.0004**	[-7.71 to -2.33]
		LE	126.14 ± 24.24	119.93 ± 6.13	0.5524	122.93 ± 17.36		**0.0001**	[-7.71 to -2.33]

Table 7. mERG values in peripheral rings and reference values

	Ring	Eye	Group 2 Non-chelated	Group 3 Chelated	p-value	Group 1 Total patients	Reference values	p-value	95%CI
Amplitude P1-N1	Ring 5–10°	RE	45.30± 8.96	38.92 ± 12.4	0.1415	42.35 ± 10.95	60.38 ± 7.65*	**0.0001**	[12.69 to 23.37]
		LE	45.8 ± 7.22	41.2 ± 12.58	0.2596	43.74 ± 10.10		**0.0001**	[11.58 to 21.70]
	Ring 10–15°	RE	46.28 ±10.06	39.2 ±14.89	0.163	43.01 ± 12.77	45.12 ± 7.88*	0.4821	-
		LE	46.7 ± 6.95	38.7 ± 14.59	0.1728	43.06 ± 11.62		0.4614	-
	Ring > 15°	RE	12.9 ± 2.48	11.7 ± 3.89	0.3513	12.36 ± 3.20	38.38 ± 7.08*	**0.0001**	[22.95 to 29.09]
		LE	13.2 ± 2.0	11.7 ± 3.82	0.2608	12.5 ± 3.0		**0.0001**	[22.84 to 28.92]

Peripheral Rings : Ring 3 = 5° – 10°; Ring 4 = 10° – 15°; and Ring 5 = > 15°
Amplitude P1-N1 (nV/deg2).