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Lead Accumulation as Possible Risk Factor for Primary Open-Angle Glaucoma

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Abstract We evaluated the association between hair lead concentrations and primary open-angle glaucoma. Ninety-eight Japanese patients (40 males, 58 females; average age 57.6± 10.8 years) with primary open-angle glaucoma and control subjects (131 males, 114 females; average age 56.0±12.8 years) were recruited in this study. Hair lead levels were measured by inductively coupled plasma mass spectrometry. Hair lead concentrations between primary open-angle glaucoma and control groups were compared using Mann–Whitney *U* test. As a subgroup analysis, we compared hair lead concentrations between low-tension glaucoma, high-tension glaucoma, and control groups using one-factor analysis of variance. Lead accumulation levels were significantly higher in the female subjects with primary open-angle glaucoma compared to the control group (P=0.03). Lead accumulation levels were significantly higher in female patients with low intraocular pressure compared to control group 2 (P=0.02). A higher hair lead level, which reflects the total body burden of lead, was observed to be associated with primary open-angle glaucoma in females especially with low-tension glaucoma. Accumulation of lead may be an unrecognized risk factor of non-pressure-dependent glaucomatous optic neuropathy.

Keywords Primary open-angle glaucoma · Lead · Hair · Inductively coupled plasma mass spectrometry · Neurotoxicity

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Introduction

Glaucoma is considered as a multifactorial disease where the intraocular pressure (IOP) is the most important risk factor in the context of progression of glaucomatous optic nerve damage. Lowering IOP may not be effective in every case since other possible mechanisms may be operating such as ischemia, obstruction of axoplasmic flow, deprivation of one or more trophic factors, and excitotoxicity.

Recently, lead has been suggested to be potentially toxic to several tissues in the urogenital, peripheral, and central nervous systems [1, 2]. Schaumberg et al. reported that there was a greater than 2.5-fold increased risk of cataract in males with higher tibial levels of lead compared to men with lower lead levels [3]. Of concern is the toxicity from lead to retinal nerves and ganglion cells leading to visual loss.

In this study, we investigated the hair tissue concentrations of lead in patients with primary open-angle glaucoma (POAG) and control subjects.

Patients and Methods

Ninety-eight consecutive Japanese patients (40 males, 58 females; average age 57.6± 10.8 years) with newly or previously diagnosed unilateral or bilateral POAG at the glaucoma subspecialty clinic of Keio University Hospital were recruited between January 2005 and March 2007 in this prospective study. The study followed the Tenets of the Declaration of Helsinki. Written informed consents about hair sampling and experimental procedures were obtained from all subjects before entry into the study. This study was approved by the Ethical Committee of the Keio University School of Medicine.

All subjects with POAG underwent a complete ophthalmic examination, including best corrected visual acuity measurements, slit lamp biomicroscopy, pentacam corneal thickness evaluation (Oculus, Wetzlar, Germany), Goldmann applanation tonometry, gonioscopy, funduscopy, and optic disk evaluation with a 90-diopter lens by a single investigator (K.Y.) with subspecialty training in glaucoma. In addition, Humphrey visual field testing was conducted with a Humphrey field analysis using the 30-2 Swedish Interactive Threshold Algorithm Standard Strategy (Zeiss, Dublin, USA). Definite primary open-angle glaucoma cases were required to meet the following criteria: (1) angles were open and not occludable in both eyes on the basis of gonioscopy and (2) visual field defects were present and consistent with glaucomatous optic neuropathy in the visual field tests. Subjects with other ophthalmic conditions, such as angle-closure glaucoma, pigment dispersion glaucoma, exfoliative glaucoma, trauma, any other type of secondary glaucoma, subjects with high myopia exceeding −10D or hyperopia above +3D, subjects below 25 or over 85 years of age, were excluded. None of the patients in the current study had a history of previous glaucoma or ocular surgery.

We divided our subjects with POAG according to the gender and the IOP levels. IOP measurements were performed seven times (at 6, 9, 12, 15, 18, 21, 24 o'clock as 24-h IOP measurements) in POAG patients. Patients whose highest IOP was below 14 mm Hg were enrolled in the low-IOP group and patients whose highest IOP was above 15 mm Hg were enrolled in the high-IOP group. The high-IOP group consisted of 23 males and 44 females and the low-IOP group consisted of 17 males and 14 females. This division was based on the mean IOP levels in Japanese POAG patients as described previously in a large epidemiological study made in Japan [4].

With the intension to conduct this research on a large number of subjects, the protocol was designed as a multicenter study. We compared the POAG group with two control

groups. Subjects of control group 1 (120 males, 95 females; average age 55.9±13.3 years) were recruited only from individuals who came to six primary ophthalmic health screening centers in the Tokyo district between January 2005 and March 2007 and diagnosed not to have glaucoma or other ocular disorders based on questionnaires which assessed diagnosis from presence of normal IOP values by pneumotonometer assessment and normal optic nerve head findings by funduscopy at the examination centers. Subjects of control group 2 (11 males, 19 females; average age 56.0±16.0 years) were recruited from patients who came to the Keio University Hospital for annual ophthalmic refractive examinations between January 2005 and March 2007. Control group 2 did not have any history of ocular diseases and underwent the same examinations as the POAG patients. Humphrey visual field testing and central corneal thickness measurement could not be performed in control group 2 because the Ethical Board Committee did not allow conduct of these test on subjects shown to be normal by IOP testing and funduscopy due to economical burden.

The systemic medications allowed in this study were those for arterial hypertension and diabetes mellitus.

Since recent literature evidence suggested that health effects of toxic metals differ in male and female, we chose to analyze the gender-specific differences according to the initial protocol of our study [5–7].

Hair Sampling and Analysis

Hair was cut adjacent to the scalp from several areas of the parieto-occipital region using clean stainless steel scissors. Hair specimens measured 3.0 cm in length, about 0.2 g in weight. Hair samples were washed using a modified method developed by the International Atomic Energy Agency [8]. First, the samples were washed with acetone, then with 0.01% Triton-X 100 (ICN Biomedicals, Costa Mesa, CA, USA), and finally with ultrapure water. Then, 2.5 ml of tetramethyl ammonium hydroxide (Tama Chemicals, Kawasaki, Japan) was added to the specimens with 15 μl of a standard multimineral solution (SPEX CertiPrep, Metuchen, USA). Finally, ultrapure water was added for a final volume of 10 ml, and the sample was shaken for 2 h at 75°C to completely digest the hair. The samples were then cooled to room temperature and topped up to 1,500×*g* (gravimetric) with ultrapure water. All of the metals in the samples were analyzed using inductively coupled plasma mass spectrometry (HP-7500I; Yokogawa, Tokyo, Japan) by the international standard method [9].

The results are expressed in part per billion (ng/g). Data were expressed as mean± standard deviation. Lead level in hair samples was quantified. Hair is a reliable biomarker of the total body tissue burden of metals and provides a picture of long-term heavy metal exposure [10, 11]. Laboratory personnel were masked to clinical and demographic information on the patients.

Statistical Analysis

Age and body mass index were compared between cases and control groups 1 by unpaired *t* test. Prevalence of diabetes mellitus and hypertension was compared by the Fisher exact test between POAG and control group 1. Aforementioned parameters were compared between the high-IOP, low-IOP, and control group 2 by one-factor analysis of variance (ANOVA) and the Fisher exact test. Hair lead concentrations between POAG and control group 1 were performed using Mann–Whitney *U* test. Hair lead concentrations among high-IOP group, low-IOP group, and control group 2 were

Table 1 Demographic characteristics and hair lead concentrations of male and female subjects in POAG and control group 1

	Male			Female		
	POAG	Controls	*P* value	POAG	Controls	*P* value
Number	40	120		58	95	
Age (years)	53.2±12.0	55.9±10.3	0.20	58.7±11.0	58.9±13.1	0.92
BMI	23.2±2.7	23.1±2.5	0.96	21.5±3.1	21.5±3.1	0.97
DM	6 (15.0%)	11 (9.2%)	0.37	5 (8.6%)	7 (7.4%)	1.00
Hypertension	8 (20.0%)	15 (12.5%)	0.30	8 (13.8%)	11 (11.6%)	0.80
Metal levels						
Lead	286.5±241.5	404.7±436.8	0.18	642.0±638.3	419.6±405.3	0.03

Values are represented at mean±standard deviation; Lead, part per billion (ppb)

POAG primary open-angle glaucoma, *BMI* body mass index, *DM* diabetes mellitus

performed using one-factor ANOVA after logarithmic transformation of heavy metal variables to achieve normal distribution. Post hoc test was done by Sheffe multiple comparisons. Central corneal thickness was compared between the high-IOP group and the low-IOP group by unpaired *t* test. Mean deviation was compared between the high-IOP group and the low-IOP group by Mann–Whitney *U* test. All the comparisons were made in male and female subjects.

A *P* value less than 0.05 was considered statistically significant. Statistical analysis was performed with the SPSS statistical program package (ver. 15.00 Chicago, IL, USA). Our preliminary study showed that the mean±standard deviation of hair lead levels in normal controls in Japanese is approximately 496.1±558.0 ppb. Thus, when the significance level

Table 2 Demographic characteristics and hair lead concentrations of male and female subjects in high-IOP, low-IOP, and control group 2

	Male				Female			
	High IOP	Low IOP	Controls	*P* value	High IOP	Low IOP	Controls	*P* value
Number	23	17	11		44	14	19	
Age (years)	57.8±8.4	54.4±11.9	54.0±17.0	0.39	59.8±9.9	56.8±12.7	60.0±16.8	0.47
BMI	24.0±2.3	22.1±2.9	23.2±3.1	0.29	21.4±3.5	21.5±2.3	21.0±2.5	0.62
DM	3 (13.0%)	2 (11.7%)	0 (0%)	0.70	3 (6.8%)	0 (0%)	0 (0%)	0.75
Hypertension	5 (21.7%)	3 (17.6%)	1 (9.0%)	0.89	4 (9.1%)	3 (21.4%)	0 (0%)	0.08
CCT	535.9±32.4	535.9±38.6		0.35	529.9±27.2	525.2±32.1		0.55
MD value	−8.2±7.2	−10.8±7.3		0.94	−4.9±4.7	−7.8±7.3		0.11
Metal levels								
Lead	311.9± 259.5	251.9± 217.5	282.9± 251.9	0.47	522.3± 508.5	903.3± 810.7	382.6± 347.2	0.02

Values are represented at mean±standard deviation (*P* value: one-factor ANOVA, *t* test, Fisher exact test). Both values were in the right eye. Lead, part per billion (ppb)

POAG primary open-angle glaucoma, *BMI* body mass index, *DM* diabetes mellitus, *CCT* central corneal thickness (μm), *MD* mean deviation

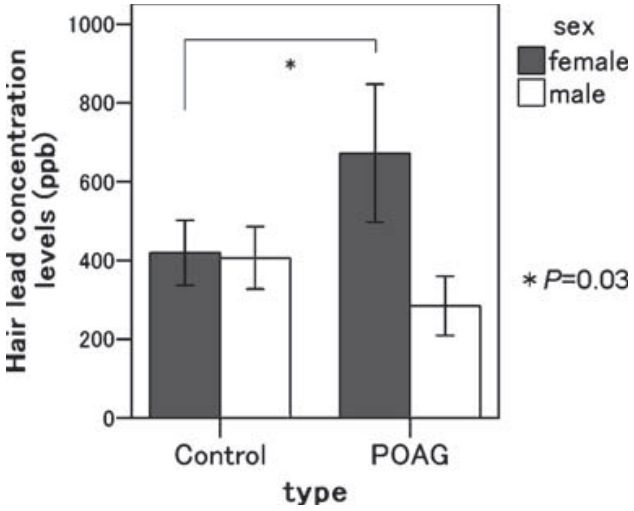

Fig. 1 The relation between lead accumulation in POAG and control group 1. Note the significantly higher level of lead accumulation in POAG group in female subjects compared to control group 1 in female subjects (P=0.03). Abbreviations: *ppb*, part per billion; *POAG*, primary open-angle glaucoma

is 0.05, this study with 40 males with glaucoma and 120 male controls and 58 females with glaucoma and 98 female controls has more than 80% power to detect 60% difference of averages.

Results

No statistical differences were found between the POAG and control group 1 in each gender in relation to age, body mass index, prevalence of diabetes mellitus and hypertension (Table 1). Likewise, no significant differences were observed between aforementioned parameters between the low-IOP, high-IOP group, and control group 2 in both males and females (Table 2)

Lead accumulation levels were significantly higher in subjects with POAG compared to control group 1 in the female subjects (P=0.03, Table 1, Fig. 1). No statistically significant difference in relation to accumulation of lead was shown between subjects with POAG and control group 1 in the male subjects (Table 1).

Lead accumulation levels were significantly higher in female patients with low IOP compared to control group 2 (P=0.02, Table 2, Fig. 2). No statistically significant differences in relation to accumulation of lead were shown in male subjects with high IOP and low IOP and control group 2 (Table 2).

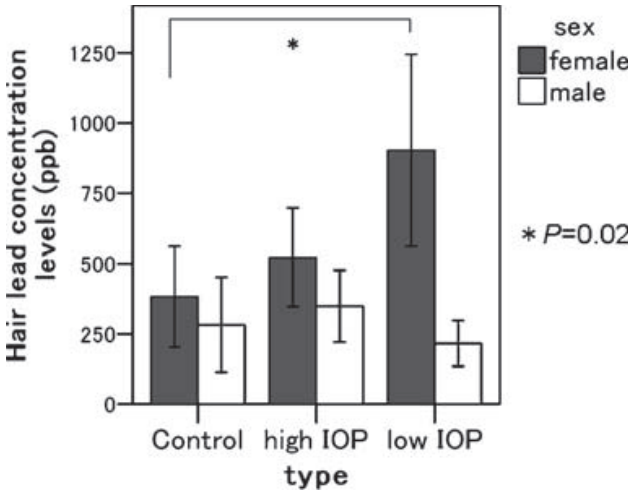

Fig. 2 The relation between lead accumulation in high-IOP, low-IOP, and control group 2. Note the significantly higher level of lead accumulation in the low-IOP group in female subjects compared to the control group in female subjects (P=0.02). Abbreviations: *ppb*, part per billion; *IOP*, intraocular pressure

Discussion

Metals such as zinc, copper, calcium, manganese, and iron have been found in ocular melanosomes, particularly within the retinal pigment epithelium [12, 13]. Heavy metals have the capacity to replace previously bound metals and change the ocular metal concentrations [14, 15]. Erie et al. reported that lead accumulated in the retinal pigment epithelium, choroid, ciliary body, and the retina of humans at concentrations greater than found in the blood [16].

Lead is known to disrupt cellular biochemistry and has profound detrimental effects on rods, bipolar cells, and photoreceptors even at very low concentrations [17, 18]. Recent evidence suggests that lead causes tissue damage by oxidative stress, resulting in lipid peroxidation, DNA damage, and the abolishment of cellular antioxidant defense mechanisms [19, 20]. Although the role of heavy-metal-induced oxidative stress in the pathogenesis of exfoliative glaucoma has been reported previously [21], there are no reports on the relation between lead accumulation and POAG.

We interestingly found a significantly higher lead level in female patients with the POAG group in our study especially in the low-IOP group. Non-pressure-related neurodegeneration may be a factor in low-tension glaucoma. Our results suggest new evidence that lead accumulation may be a possible risk factor for glaucomatous optic neuropathy without IOP elevation in female subjects. *N*-methyl-D-aspartate receptor is an ionotropic receptor for glutamate. Activation of *N*-methyl-D-aspartate receptor on the ganglion cell membrane results in the opening of a Ca channel which allows flow of small amounts of Ca^{2+} ions into the ganglion cell. The Ca influx through *N*-methyl-D-aspartate receptor is associated with glaucomatous optic neuropathy by its exocytotoxicity [22]. Several reports of exposure to lead in neuronal cell indicate disruption of the *N*-methyl-D-aspartate receptor ionophore [23, 24] and it may induce the ganglion-cell-specific apoptosis induced by lead.

Sex-related differences in relation to lead accumulation have not been clarified in the literature. Efflux of lead from bone in females has been reported to occur with age [25], pregnancy [26], lactation, and menopause [25, 27] and has been proposed as a possible mechanism for the gender-related accumulation differences. The rate of progression of optic nerve damage has been reported to be shorter in females with low-tension glaucoma compared to males with low-tension glaucoma [28].These differences may very well be explained with a high level of lead accumulation in ocular tissues in females compared to males.

The National Health and Nutrition Examination Survey report suggested that blood lead levels could increase as much as 25% in the 5 years following menopause [29]. Since all female subjects were perimenopausal or postmenopausal in our study, the high hair levels of lead may be explained by the aforementioned sex-related physiological differences.

This study may have certain limitations. The study did not establish causal relationship based on histopathology. Future histological or ultrastructural studies of the optic nerve samples obtained from consenting patients within a few hours of death will also enrich our understanding on tissue-related effects of heavy metals. Another limitation is that perimetry could not be performed in control subjects according to Ethical Board Committee decision. Further studies deliminating the sensory deficit differences between patients with POAG and control subjects and hair lead concentrations should be conducted. The subjects of the current prospective study were recruited consecutively without randomization which might be a source of further bias. Future randomized studies will clarify unresolved issues emphasized in our study.

In summary, accumulation of lead may be an unrecognized risk factor for glaucomatous optic neuropathy. The potential toxicity of heavy metal in glaucomatous nerve damage requires further studies.

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Competing interests None

Fundings None

References

1. Kim R, Rotnitsky A, Sparrow D, et al (1996) A longitudinal study of low-level lead exposure and impairment of renal function. The normative aging study. JAMA 275:1177–1181.
2. Needleman HL, Schell A, Bellinger D, et al (1990) The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. N Engl J Med 322:83–88.
3. Schaumberg DA, Mendes F, Balaram M, et al (2004) Accumulated lead exposure and risk of age-related cataract in men. JAMA 292:2750–2754.
4. Iwase A, Suzuki Y, Araie M, et al (2004) The prevalence of primary open-angle glaucoma in Japanese: the Tajimi Study. Ophthalmology 111:1641–1648.
5. Vahter M, Akesson A, Liden C, et al (2007) Gender differences in the disposition and toxicity of metals. Environ Res 104:85–95.
6. Pirkle JL, Kaufmann RB, Brody DJ, et al (1998) Exposure of the U.S. population to lead, 1991–1994. Environ Health Perspect 106:745–750.
7. Barregard L, Svalander C, Schutz A, et al (1999) Cadmium, mercury, and lead in kidney cortex of the general Swedish population: a study of biopsies from living kidney donors. Environ Health Perspect 107:867–871.
8. Puchyr RF, Bass DA, Gajewski R, et al (1998) Preparation of hair for measurement of elements by inductively coupled plasma-mass spectrometry (ICP-MS). Biol Trace Elem Res 62:167–182.
9. Rodushkin I, Axelsson MD (2000) Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails. Part I. Analytical methodology. Sci Total Environ 250:83–100.
10. Goulle JP, Mahieu L, Castermant J, et al (2005) Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair. Reference values. Forensic Sci Int 153:39–44.
11. Saltzman BE, Gross SB, Yeager DW, et al (1990) Total body burdens and tissue concentrations of lead, cadmium, copper, zinc, and ash in 55 human cadavers. Environ Res 52:126–145.
12. Potts AM, Au PC (1976) The affinity of melanin for inorganic ions. Exp Eye Res 22:487–491.
13. Panessa BJ, Zadunaisky JA (1981) Pigment granules: a calcium reservoir in the vertebrate eye. Exp Eye Res 32:593–604.
14. Sarna T, Hyde JS, Swartz HM (1976) Ion-exchange in melanin: an electron spin resonance study with lanthanide probes. Science 192:1132–1134.
15. Jamall IS, Roque H (1989) Cadmium-induced alterations in ocular trace elements. Influence of dietary selenium and copper. Biol Trace Elem Res 23:55–63.
16. Erie JC, Butz JA, Good JA, et al (2005) Heavy metal concentrations in human eyes. Am J Ophthalmol 139:888–893.
17. Goyer RA (1993) Lead toxicity: current concerns. Environ Health Perspect 100:177–187.
18. Fox DA, Srivastava D, Hurwitz RL (1994) Lead-induced alterations in rod-mediated visual functions and cGMP metabolism: new insights. Neurotoxicology 15:503–512.
19. Hsu PC, Guo YL (2002) Antioxidant nutrients and lead toxicity. Toxicology 180:33–44.
20. Ahamed M, Siddiqui MK (2007) Low level lead exposure and oxidative stress. Current opinions. Clin Chim Acta 383:57–64.
21. Yildirim Z, Ucgun NI, Kilic N, et al (2007) Pseudoexfoliation syndrome and trace elements. Ann N Y Acad Sci 1100:207–212.
22. Sucher NJ, Lipton SA, Dreyer EB (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. Vision Res 37:3483–3493.
23. Marchetti C, Gavazzo P (2005) NMDA receptors as targets of heavy metal interaction and toxicity. Neurotox Res 8:245–258.

24. Busselberg D, Michael D, Platt B (1994) Pb2+reduces voltage- and *n*-methyl-*d*-aspartate (NMDA)-activated calcium channel currents. Cell Mol Neurobiol 14:711–722.
25. Webber CE, Chettle DR, Bowins RJ, et al (1995) Hormone replacement therapy may reduce the return of endogenous lead from bone to the circulation. Environ Health Perspect 103:1150–1153.
26. Gulson BL, Jameson CW, Mahaffey KR, et al (1997) Pregnancy increases mobilization of lead from maternal skeleton. J Lab Clin Med 130:51–62.
27. Symanski E, Hertz-Picciotto I (1995) Blood lead levels in relation to menopause, smoking, and pregnancy history. Am J Epidemiol 141:1047–1058.
28. Drance S, Anderson DR, Schulzer M (2001) Risk factors for progression of visual field abnormalities in normal-tension glaucoma. Am J Ophthalmol 131:699–708.
29. Silbergeld EK, Schwartz J, Mahaffey K (1988) Lead and osteoporosis: mobilization of lead from bone in postmenopausal women. Environ Res 47:79–94.