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Levels of zinc, iron, and copper in patients with pseudoexfoliative cataract

T. CUMURCU[1], D. MENDIL[2], I. ETIKAN[3]

[1]School of Medicine, Department of Ophthalmology
[2]School of Science and Arts, Department of Chemistry
[3]School of Medicine, Department of Biostatistic, Gaziosmanpasa University, Tokat - Turkey

PURPOSE. *To investigate the aqueous humor, lens, and serum concentrations of zinc, iron, and copper in patients with pseudoexfoliative cataract and compare with patients who have senile cataract without pseudoexfoliation.*
METHODS. *Twenty-five patients with pseudoexfoliative cataract and 25 patients with senile cataract as control group were enrolled in the study. Samples from aqueous humor, serum, and lens materials during extracapsular cataract extraction (ECCE) were collected from all patients. The levels of selected trace elements in three samples in all groups were assayed with atomic absorption spectrometry (AAS) and statistical analyses were performed with* t*-test for independent samples except lens zinc and serum copper levels. The data weren't normally distributed, therefore Mann-Whitney* U *test applied for these parameters.*
RESULTS. *The zinc and copper levels in aqueous humor of PEX group were significantly higher than those of control group ($p<0.001$). The iron levels in aqueous humor were not significantly different in PEX group and control group ($p=0.252$). The copper content of lenses was significantly increased in PEX group compared to control group ($p=0.029$). The iron and zinc content of lenses had no significant differences between the two groups ($p=0.248$, $p=0.719$, respectively). The levels of iron and copper in serum were significantly increased in PEX group compared to control group ($p<0.001$ and $p<0.002$, respectively). The zinc level in serum had no significant differences between the two groups ($p=0.823$, $p=0.472$, respectively).*
CONCLUSIONS. *Zinc, iron, and especially copper may play a role in PEX syndrome. (Eur J Ophthalmol 2006; 16: 548-53)*

KEY WORDS. *Aqueous humor, Atomic absorption spectrometry, Lens, Pseudoexfoliative cataract, Serum, Trace element*

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INTRODUCTION

Pseudoexfoliation (PEX) syndrome is an age-related disorder of unknown etiology, which is characterized by the abnormal production and accumulation of a fibrillar extracellular material in many intraocular and extraocular tissues including skin and connective tissue portions of various visceral organs (1, 2).

Although the exact composition of the abnormal material is not known, immunohistochemical evidence suggests a complex glycoprotein/proteoglycan-structure composed of non-collagenous basement membrane components, elastic fiber components, and carbohydrate groups (3, 4). The basement membrane components heparan and chondroitin sulphate proteoglycan, and entactin/nidogen and particularly the elastic fiber components elastin, amyloid P, vitronectin, and fibrillin-1, are integral constituents of PEX material (4, 5). On the other hand, some studies performed on the nature of PEX material revealed an association with amyloid (6, 7).

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Recently, electron microscopic and biochemical studies have demonstrated composition of PEX material (8-11). Some authors have shown the level of trace elements in cataractous lenses. They have compared the level of trace elements in diabetic and nondiabetic lenses and types of cataracts but the same levels have not been studied in PEX syndrome (12, 13). With the help of these studies, we aimed to show the iron, zinc, and copper levels in serum, aqueous humor, and cataractous lenses of patients with PEX and without PEX using atomic absorption spectrometry (AAS) method.

METHODS

This study was conducted on a total of 50 patients. The study group consisted of 25 cases of cataract in eyes with pseudoexfoliation. The control group consisted of 25 cases of cataract in eyes without pseudoexfoliation. All experiments were performed according to the Declaration of Helsinki. All subjects gave informed consent as approved by local medical ethics committee.

The mean age of the PEX group was 63.08 ± 8.6 years (49 to 79 years) (17 female/8 male) and the control group was a mean age of 62.32 ± 7.5 years (50 to 80 years) (16 female/9 male).

Human aqueous humor, lens, and serum samples were taken in the operation room during cataract surgery, which was performed with extracapsular technique. The cortex and nuclear parts of the lenses were used for analysis. There were nuclear (n=14) and cortical (n=11) cataract types in both groups. All subjects had not given a history of systemic medical disease and drug usage. Mature, metabolic, and secondary cataract types were excluded from the study. Patients whose visual acuity was 60/200 or worse preoperatively were included in the study.

After a night fasting, 5 cc blood samples of all patients were obtained. The materials were centrifuged and stored in –20 °C until analyzed. Lens materials were homogenized and with 0.1 mL aqueous humor, taken during surgery, were studied with AAS at –20 °C.

Reagents

Reagent quality deionized water (18.2 M cm) from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all aqueous solutions. All mineral acids and oxidants (HNO_3 and H_2O_2) used were of the highest quality (Suprapure, Merck, Darmstadt, Germany). All plastic and glassware containers were cleaned by soaking with the contact overnight in a 10% (w/v) nitric acid solution and then rinsed with deionized water.

Analytical procedure

The blood samples were centrifuged at 2400 rpm for 15 minutes to separate the serum. Samples were kept in a freezer at –20 °C until analysis.

A total of 1.0 mL sample of serum was digested with 3 mL of HNO_3 (65%), 1 mL of H_2O_2 (30%) in a microwave digestion system for 31 minutes and diluted to 5 mL with deionized water. The 0.1 g sample of the lens were digested with 2 mL of HNO_3 (65%), 0.5 mL of H_2O_2 (30%) in a microwave digestion system for 31 minutes and diluted to 5 mL with deionized water. Aqueous humor (0.1 mL) was diluted to 3 mL with deionized water. A blank digest was carried out in the same way (digestion conditions for microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min). This procedure was preferred because it is more accurate with respect to both time and recovery values. The recovery values were nearly quantitative (>95%) for this digestion method (14).

In this study, a Perkin Elmer AA analyst model 700 atomic absorption spectrometer with deuterium background corrector was used. Cu, Zn, and Fe levels in the serum, lens, and aqueous humor samples were determined using a flame atomic absorption spectrometry (FAAS) graphite furnace using argon as an inert gas. Pyrolytic-coated graphite tubers with a platform were used and signals were measured at their peak. Cu, Zn, and Fe levels were measured at 324.8 nm, 213.9 nm, and 248.3 nm wavelengths, respectively (15).

All the measurements were taken twice. Results were calculated in terms of μmol/g wet tissue weight. Iron, zinc, and copper were measured in lens, aqueous humor, and serum, respectively (Tabs. I–III).

All analyses were performed by the same examiners who were not aware which group was being examined. All samples were assayed in duplicate. Continuous data are expressed as mean ± standard deviation and categorical data as counts and percentages.

Except lens zinc and serum copper levels, for the statistical analysis of the other parameters between two groups, we used *t*-test for independent samples. As the lens zinc and serum copper levels were not distributed normally (used with Levene's test for equality of variance), the nonparametric Mann-Whitney *U* test was used in their statistical analysis. A two-tailed probability value of $p<0.05$ was regarded as statistically significant. All data were analyzed using SPSS for Windows, version 11.5.

RESULTS

There was no statistical difference between the mean ages of the groups (p=0.742). The zinc and copper levels in aqueous humor of PEX group was significantly higher than those of control group ($p<0.001$) (aqueous humor zinc 16.31 ± 5.28 vs 8.14 ± 4.36 and aqueous humor copper 12.94 ± 4.45 vs 8.14 ± 2.72 μmol/g wet tissue). The iron level in aqueous humor was not significantly different in PEX group and control group (p=0.252) (aqueous humour iron 30.21 ± 8.2 vs 27.31 ± 9.1 μmol/g wet tissue) (Tab. I).

The copper content of lenses was significantly increased in PEX group compared to control group (p=0.029) (lens copper 0.028 ± 0.09 vs 0.021± 0.11 μmol/g wet tissue). The iron and zinc content of lenses had no significant differences between the two groups (p=0.248, p=0.719) (lens iron 0.55 ± 0.14 vs 0.51 ± 0.1 μmol/g wet tissue, lens zinc 0.44 ± 0.6 vs 0.40 ± 0.14 μmol/g wet tissue) (Tab. II).

The levels of iron and copper in serum were significantly increased in PEX group compared to control group ($p<0.001$ and $p<0.002$, respectively) (serum iron 66.58 ± 11.83 vs 45.33 ± 13.69 μmol/g wet tissue and serum copper 12.88 ± 2.91 vs 15.75 ± 5.71 μmol/g wet tissue). The zinc level in serum had no significant differences between the two groups (p=0.823) (serum zinc 16.11 ± 3.71 vs 16.46 ± 6.74 μmol/g wet tissue) (Tab. III).

DISCUSSION

Previous reports support that PEX syndrome is a systemic disorder and the origin of the PEX material is unknown. The aim of the present studies was to evaluate composition of abnormal PEX material, which is multifocally produced by various cell types of the anterior segment of the eye (16).

AAS is a highly sensitive method for research of organic materials with unknown composition, such as PEX material

TABLE I - TRACE ELEMENT LEVELS IN LENSES IN PEX AND CONTROL GROUP

	Lens (PEX)	Lens (control)	p Value
Iron (Fe)	0.55 ± 0.14	0.51 ± 0.10	0.248
Zinc (Zn)	0.44 ± 0.60	0.40 ± 0.14	0.472
Copper (Cu)	0.28 ± 0.09	0.21 ± 0.11	0.029*

Values are μmol/g; *Significant; PEX = Pseudoexfoliative cataract

TABLE II - TRACE ELEMENT LEVELS IN AQUEOUS HUMOR IN PEX AND CONTROL GROUP

	Aqueous humor (PEX)	Aqueous humor (control)	p Value
Iron (Fe)	30.21 ± 8.20	27.31 ± 9.1	0.252
Zinc (Zn)	16.31 ± 5.28	8.14 ± 4.36	0.001*
Copper (Cu)	12.94 ± 4.45	8.14 ± 2.72	0.001*

Values are μmol/g; *Significant; PEX = Pseudoexfoliative cataract

TABLE III - TRACE ELEMENT LEVELS IN SERUM IN PEX AND CONTROL GROUP

	Serum (PEX)	Serum (control)	p Value
Iron (Fe)	66.58 ± 11.83	45.33 ± 13.69	0.001*
Zinc (Zn)	16.11 ± 3.71	16.46 ± 6.74	0.823
Copper (Cu)	15.75 ± 5.71	12.88 ± 2.91	0.02*

Values are μmol/g; *Significant; PEX = Pseudoexfoliative cataract

(17). In the determination of trace metal ions for biological samples, AAS is generally the main instrument (14, 15).

Schlötzer-Schrehardt et al demonstrated the presence of many elements both in mature and aggregating PEX fibrils of the lens capsule by using energy-filtering transmission electron microscopy (EFTEM). The method is used to detect elements and mapping on the lens surface (8).

In our study, some trace element levels were measured in aqueous humor, lens, and serum with method of AAS. Because PEX is a systemic disorder and a simultaneous mechanism of local production of the material by anterior segment tissues and transport via the aqueous humor has been suggested, measurement of trace element levels must be performed not only in lens material but also in aqueous humor and serum (18, 19).

Dawczynski et al evaluated zinc and iron levels of lenses in different types of cataracts. This study demonstrated a strong relationship between lens color, lens maturity, and increased levels of iron and zinc in the lens (12). Another study showed that the zinc concentration in diabetic cataractous lenses was significantly increased compared to the nondiabetic cataractous lenses (13). Because of that we excluded the diabetic patients and mature cataractous lenses in this study. Similar studies were reported about the role of trace elements in senile and diabetic cataract (20, 21).

Although there is a relationship between cataract and PEX syndrome, there is no report about levels of trace elements in PEX syndrome (22).

There are many studies related to pathogenesis of PEX. Veroman et al reported that excessive sucrose and salt consumption can trigger crystallin leakage from the lens and PEX formation (11). Schlötzer-Schrehardt et al reported that the zinc signal in PEX fibrils may be associated with the presence of zinc-dependent enzymes, such as matrix metalloproteinases, which have been shown to accumulate within PEX material by immunohistochemistry and have been suggested to play a role in the aberrant matrix metabolism (23). Koliakos et al reported on aqueous composition in PEX and have provided evidence that oxidative stress may play role in pathogenesis of PEX. They demonstrated lower concentration of ascorbic acid in aqueous humor of patients with PEX. Ascorbic acid is the major antioxidant in the eye (24). Also they reported that the aqueous level of 8-isoprostaglandine $F_2\alpha$ increased in PEX more than in control samples. 8-isoprostaglandine $F_2\alpha$ is a marker of oxidative stress (25).

We have also investigated the role of some trace elements in PEX syndrome because some trace elements were found to be higher in cataractous lenses than healthy controls (21, 26). Therefore, the levels of trace elements are important in patients with PEX due to relationship between PEX and cataract (22).

Dawczynsky et al reported significantly increased levels of zinc in cataractous lenses compared to control subjects. Similarly, Gündüz et al reported significantly increased levels of zinc in diabetic lenses (12, 13). Increased zinc levels may disturb the permeability of the lens membrane by oxidation (21). In our study zinc levels increased in lenses in the PEX group but not significantly. The aqueous levels of Zn were higher in the PEX group than the control group. Zinc is normally found in intraocular tissues and aqueous humor and has been suggested to play a role in various pathologic conditions such as Alzheimer disease by interacting with and aggregating proteins such as amyloid precursor protein and ß-amyloid (27). The material of PEX might be amyloid structure as reported previously (6, 7).

Schlötzer-Schrehardt et al demonstrated irregular punctate zinc signals on intracapsular PEX fibers (8). The amyloid material was shown in aqueous humor previously (27). Therefore, Zn might be aggregate like APP and beta amyloid structures in Alzheimer disease. In some studies, it was demonstrated that the levels of Zn in senile cataractous lenses were elevated but there is no report of levels of Zn in aqueous humor with cataractous lenses or PEX (12, 25).

We found that the Cu levels increased significantly in lens, aqueous humor, and serum in the PEX group. In some previous studies, higher levels of Cu have been detected in human cataractous lenses compared to healthy subjects (28, 29). Ortwerth and James demonstrated that addition of lens proteins to mixture of copper and ascorbate suppresses its oxidation and free radical generation. Therefore, when Cu increases in cataract, it would be bound by proteins of lens (30). Saxena at al suggest that chelation therapy may be useful for evaluating the role of protein-bound Cu in senile cataractogenesis, which also aids the delay of process (31). The increased concentration of Cu in the lens, serum, and aqueous humor of PEX patients demonstrated that Cu might have a role in matrix formation of PEX material.

In previous studies, increased Fe level was demonstrated in cataractous lens (26, 28). Fe undergoes enzymatic and nonenzymatic oxidation and peroxidation of biological molecules. Ferrous iron yields superoxide radicals by giving its electron to molecular oxygen and becomes Fe^{3+}

(Fenton reaction). Superoxide dismutase results in superoxide radicals to H_2O_2, which may be converted into H_2O or into the hydroxyl radical OH spontaneously. Accumulation of hydroxyl radical or ferryl ion may exist in cataractous lenses (12, 26). We found increased aqueous and lens levels of Fe in PEX, but differences were not significant. The serum levels of Fe were significantly higher in PEX group than the control group.

Further studies should be performed to demonstrate whether some trace elements play a role in cataract in eyes with pseudoexfoliation.

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Reprint requests to:
Tongabay Cumurcu, MD
Gaziosmanpasa University
School of Medicine
Tokat, Turkey 61100
tongabay@superonline.com
tcumurcu@gop.edu.tr

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