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https://doi.org/10.31925/farmacia.2024.5.15 **ORIGINAL ARTICLE**

HAIR AND SERUM TRACE ELEMENT AND MINERAL LEVELS IN PATIENTS WITH AGE-RELATED CATARACT

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Abstract

The objective of the present study was to assess hair and serum trace element levels in patients with age-related cataracts and healthy controls with adjustments for the presence of other comorbidities. A total of 121 adults, including 61 patients with age-related cataracts and 60 subjects without eye diseases, were examined. Age, sex, body mass index and other comorbidities were registered. Serum and hair essential trace elements (Co, Cr, Cu, Fe, I, Li, Mn, Mo, Se, V, Zn) and mineral (Mg, Ca) levels were evaluated using inductively coupled plasma mass spectrometry. The obtained data demonstrate that circulating Mn, Se, V and Zn levels in patients with cataracts were 9% (p = 0.009), 10% (p = 0.001), 15% (p = 0.003) and 7% (p = 0.015) lower when compared to the subjects without eye pathology. In hair only, the Zn level in patients with cataracts was significantly 13% (p = 0.005) lower when compared to eye disease-free controls. In serum and hair samples, the levels of Co were significantly lower in cataract patients than in controls by 16% (p = 0.068) and 25% (p = 0.062), respectively. Multiple linear regression demonstrated that after adjustment for age, sex, BMI and the presence of other comorbidities, only serum Se and Zn levels were significantly associated with a diagnosis of cataract. The obtained data demonstrate that low serum zinc, especially selenium levels, are independently associated with an increased risk of cataracts.

Rezumat

Obiectivul studiului a fost de a evalua nivelurile de oligoelemente din păr și ser la pacienții cu cataractă asociată vărstei. Au fost examinați un total de 121 de adulți, dintre care 61 de pacienți cu cataractă și 60 de voluntari sănătoși fără patologii oculare. Au fost înregistrate vârsta, sexul, indicele de masă corporală și alte comorbidități. Nivelurile de oligoelemente esențiale din ser și păr (Co, Cr, Cu, Fe, I, Li, Mn, Mo, Se, V, Zn) și minerale (Mg, Ca) au fost evaluate utilizând spectrometria de masă cu plasmă cuplată inductiv. Datele obținute demonstrează că nivelurile circulante de Mn, Se, V și Zn la pacienții cu cataractă au fost cu 9% (p = 0,009), 10% (p = 0,001), 15% (p = 0,003) și 7% (p = 0,015) mai scăzute comparativ cu subiecții fără patologie oculară. Numai în păr, nivelul de Zn la pacienții cu cataractă a fost semnificativ cu 13% (p = 0,005) mai scăzut în comparație cu grupul control fără afecțiuni oculare. În probele de ser și păr, nivelurile de Co au fost semnificativ mai scăzute la pacienții cu cataractă cu 16% (p = 0,068) și, respectiv, 25% (p = 0,062). Regresia liniară multiplă a demonstrat că, după ajustarea variabilelor vârstă, sex, IMC și prezența altor comorbidități, numai nivelurile serice de Se și Zn au fost asociate semnificativ cu diagnosticul de cataractă. Datele obținute demonstrează că nivelurile scăzute de zinc seric și, în special, de seleniu, sunt asociate independent cu un risc crescut de cataractă.

Keywords: age-related cataract, comorbidity, eye diseases, zinc, selenium

Introduction

Cataract, opacification of the eye lens, is the leading cause of vision loss [1]. Despite various types of cataracts, including childhood and secondary cataracts, age-related cataracts are the most common type of the disease [2]. The most recent estimates demonstrate that 17 million and 83.5 million cases of blindness and moderate or severe visual impairment are due to cataracts [3]. The rate of cataract-induced visual impairments was the lowest in high-income countries and the highest in South and Southeast Asia and Oceania [4]. Although accumulation of oxidatively damaged proteins in the lens due to exposure to UV light is the key factor of senile cataract development [5], genetic and environmental factors underlie susceptibility to age-related cataract development [6]. Metabolic and

lifestyle factors like obesity, type 2 diabetes mellitus, hypertension and smoking also increase the risk of age-related cataract development [7].
Nutrition and especially micronutrient intake significantly modulates age-related cataract development [8]. Specifically, it has been demonstrated that the intake of lutein, zeaxanthin, vitamins C and E, eicosapentaenoic and docosahexaenoic acids, as well as zinc and copper, may be considered protective against cataracts [9]. Trace elements like zinc [10], selenium [11] and iron [12], as well as minerals like magnesium [13] are involved in eye physiology. Certain studies demonstrate that cataract development was shown to be associated with a deficiency of zinc [14], selenium [15] and magnesium [16].
Furthermore, environmental pollution was also considered a significant risk factor for cataract development [17]. Specifically, ambient air pollution was shown to be associated with an increased risk of cataracts among adult Koreans [18]. Certain studies demonstrated that cadmium exposure is associated with an increased risk of cataracts [19]. In addition to heavy metals, urinary levels of Mg, Cr, Mn and Se were also associated with the development of age-related cataracts [20]. These findings indicate that overaccumulation of even essential trace elements may also contribute to an increased risk of cataracts, as demonstrated by increased cataractogenesis upon selenite overexposure [21] or impaired iron metabolism [22].
In view of the potential role of altered trace element and mineral metabolism in the development of metabolic and cardiovascular diseases or its comorbidity [23, 24], as well as the role of the latter as the potential factors contributing to the risk of age-related cataract development [25], it is questionable whether the association between cataract and trace element and mineral disorders is independent or mediated by other health-related risk factors.
Therefore, the objective of the present study was to assess hair and serum trace element levels in patients with age-related cataracts and evaluate the revealed associations with adjustments for the presence of other comorbidities.

Materials and Methods

The study followed the ethical principles of the Declaration of Helsinki and its later amendments. Ethical approval was obtained from the Local Ethics Committee of the Institute of Bioelementology (Orenburg State University, Russia). Recruitment of both cases and controls was performed voluntarily at the clinical centre of the ANO Centre for Biotic Medicine, Moscow, Russian Federation between 2019 and 2023. All examinees signed the informed consent form before being included in the study. All personal data and the samples collected within the study were confidentially stored and analysed in Russian research centres.

Study design

A total of 61 patients (27 men and 34 women) with age-related cataracts and 60 subjects without eye diseases (25 men and 35 women) of the respective ages were involved in the present study (Table I). Data on the diagnosis of age-related cataracts, comorbid eye diseases and other comorbidities were extracted from the case history as ICD-10 codes (Table I). In addition, body weight and height were also assessed with subsequent calculation of body mass index (BMI) using the standard formula. In view of significant variability in age, sex and BMI values, all comparisons were adjusted for these covariates. Current smoking status was also registered. To avoid the impact of side factors on the outcome of the study, the following exclusion criteria were used: specific dietary patterns (vegetarian); excessive alcohol consumption; administration of trace element and mineral-containing supplements; the presence of metallic implants, including dental amalgams or artificial joints; acute infectious diseases or traumas in less than 1 year; occupational exposure to heavy metals or involvement into heavy industry.

Reagents and equipment

9 mL Vacuette® tubes (Greiner Bio-One International AG, Austria); 1-Butanol (Merck KGaA, Darmstadt, Germany), Triton X-100 (Sigma-Aldrich, Co., St. Louis, MO USA) and concentrated nitric acid (HNO_3) (Sigma-Aldrich, Co., St. Louis, MO, USA); distilled deionised water (18.2 MΩ · cm, Labconco Corp., USA); Berghof SpeedWave-4 DAP-40 system (Berghof Products + Instruments GmbH, Germany); NexION 300D (Perkin Elmer Inc., Shelton, CT, USA) equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA); Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT, USA); Yttrium (Y) Pure Single-Element Standard (PerkinElmer Inc. Shelton, CT, USA); Rhodium (Rh) Pure Single-Element Standard (PerkinElmer Inc. Shelton, CT, USA); certified reference hair sample GBW09101 (Shanghai Institute of Nuclear Research, Shanghai, China); ClinChek® Plasma Control (Levels I, II, Lot 1286, RECIPE Chemicals + Instruments GmbH, Munich, Germany).

Sampling

A certified nurse collected venous blood and hair samples. Briefly, venous blood was collected quickly from the cubital vein using Vacuette®. Blood samples were centrifugated at 1600 rpm for 10 min to separate and collect haemolysis-free serum. The obtained serum samples were stored frozen in Eppendorf tubes until analysis. Hair sampling was performed after washing the examinees' hair with their routinely used shampoos. The proximal 1 - 2 cm of occipital scalp hair strands (0.05 - 0.1 g) less prone to external contamination were collected using ethanol-precleaned stainless steel scissors. The hair samples were stored at room temperature in paper envelopes until analysis.

Table I

Anthropometric parameters and comorbidities registered in the examinees

Parameter	ICD-10 Code	Control	Cataract
Age, years old	-	62.5 ± 10.2	65.6 ± 12
Height, cm	-	169.7 ± 9.4	168.9 ± 8.9
Weight, kg	-	77 ± 16.2	76.4 ± 15.5
BMI	-	26.7 ± 5	26.8 ± 5
Comorbidities, %			
Glaucoma	H40	0	15
Other retinal diseases	H35	0	7
Disorders of refraction and accommodation	H52	0	35
Hypertension	I10	46	57
Hypotension	I95	26	28
Angina pectoris	I20	5	22
Atherosclerosis	I70	3	22
Varicose veins of lower extremities	I83	13	20
Phlebitis and thrombophlebitis	I80	15	12
Type 2 diabetes mellitus	E11	10	5
Thyroid disorders	E00-E07	30	27
Diseases of upper respiratory tract	J30-39	13	23
Fatty liver	K76.0	11	25
Gastritis and duodenitis	K29	8	23
Other diseases of pancreas	K86	7	15
Cholelithiasis	K80	3	13
Chronic cholecystitis	K81.1	13	12
Functional intestinal disorders	K59	8	12
Xerosis cutis	L85.3	11	33
Nail diseases	L60	2	15
Androgenetic alopecia	L64	13	13
Other disorders of pigmentation	L81	7	12
Spinal osteochondrosis	M42	31	38
Osteoarthritis, unspecified site	M19.9	3	23
Osteoarthritis of hip and knee	M16, M17	20	42
Osteoporosis unspecified	M81.9	5	15
Rheumatoid arthritis seropositive	M05	10	5
Malaise and fatigue	R53	49	58
Nervousness	R45	11	25
Abnormalities of heart beat	R00	2	23
Dizziness and giddiness	R42	7	10
Other headache syndromes	G44	13	33
Sleep disorders	F51	18	25
Unspecified allergy	T78.4	5	13
Other diseases	-	43	55

Data are expressed as mean and the respective standard deviation or the relative number of examinees (%)

Preanalytical treatment

Prior to analysis, serum samples were gradually refrozen at room temperature and subsequently diluted (1:15 v/v) with a diluent containing 1% 1-Butanol, 0.1% Triton X-100 and 0.07% HNO_3 in distilled deionised water (pH = 2.0).

The obtained hair strands were washed with acetone and subsequently rinsed with distilled deionised water three times to remove dust and other potential external contamination. The washed samples were subsequently dried to a stable weight under exhaust ventilation at 60°C. Microwave-assisted acidic digestion was performed in Teflon tubes containing concentrated nitric acid in the Berghof SpeedWave-4 microwave system for 20 min (peak temperature: 170 - 180°C). Subsequently, the obtained digests were adjusted to a final volume of 15 mL with distilled deionised water.

Trace element and mineral analysis

Analysis of trace element (Co, Cr, Cu, Fe, I, Li, Mn, Mo, Se, V, Zn) and mineral (Mg, Ca) levels in the obtained samples of hair and serum was performed by inductively coupled plasma mass spectrometry (ICP-MS) at NexION 300D spectrometer equipped with autosampler. The Universal Data Acquisition Standards Kits provided by the manufacturer were used for system calibration. The 10 μg/L solutions of yttrium-89 and rhodium-103 were used for internal online standardisation. Laboratory quality control was constantly performed using the certified reference materials of human hair (GBW09101) and human plasma (ClinChek®

Plasma Control). The recovery rates for the certified reference hair and plasma materials for all elements analysed varied between 88 - 111% and 92 - 108%, respectively.

Statistical analysis

Statistical treatment of the obtained data was performed using Statistica 10.0 software (Statsoft, OK, USA). Given the skewed data distribution on hair and serum trace element and mineral levels, as evidenced by Shapiro-Wilk analysis, medians and the respective interquartile range boundaries were used as descriptive statistics. Group comparisons were performed using analysis of covariance (ANCOVA) with adjustment for sex, age and BMI values and subsequent Bonferroni post-hoc analysis after log-transformation of the raw data. Multiple linear regression was performed to evaluate the association between the level of trace elements and minerals and cataracts with adjustment for age, sex, BMI and other comorbidities. The significance level for all statistical tests was set as $p < 0.05$.

Results and Discussion

The obtained data demonstrate that the levels of trace elements and minerals in the serum of the examinees are associated with cataracts (Table II). Specifically, circulating Mn, Se, V and Zn levels in patients with cataracts were 9%, 10%, 15% and 7% lower compared to subjects without eye pathology. In turn, serum Co levels in the examined patients were 16% lower when compared to the examinees without cataracts, although this difference only tended to be lower.

Table II

Serum trace element and mineral levels (µg/mL) in patients with cataract and subjects without eye diseases

Element	Control	Cataract	P value
Ca	102.1 (96.4 - 105.4)	104 (96.5 - 109.1)	0.174
Co	0.0006 (0.0005 - 0.0007)	0.0005 (0.0004 - 0.0007)	0.068
Cr	0.0023 (0.0012 - 0.0027)	0.0018 (0.0013 - 0.0026)	0.564
Cu	1.157 (0.972 - 1.267)	1.141 (1.042 - 1.369)	0.104
Fe	1.48 (1.211 - 1.69)	1.382 (1.087 - 1.715)	0.186
I	0.064 (0.057 - 0.067)	0.064 (0.056 - 0.074)	0.358
Li	0.095 (0.078 - 0.125)	0.108 (0.076 - 0.137)	0.252
Mg	22.2 (21.2 - 23.4)	22 (21.1 - 23.8)	0.869
Mn	0.0022 (0.0019 - 0.0029)	0.002 (0.0017 - 0.0024)	0.009 *
Mo	0.0011 (0.0008 - 0.0014)	0.0011 (0.0009 - 0.0013)	0.731
Se	0.112 (0.099 - 0.133)	0.101 (0.09 - 0.112)	0.001 *
Sr	0.04 (0.031 - 0.048)	0.046 (0.036 - 0.059)	0.300
V	0.006 (0.0051 - 0.0082)	0.0051 (0.0024 - 0.0063)	0.003 *
Zn	0.972 (0.903 - 1.048)	0.901 (0.82 - 1.041)	0.015 *

Data are expressed as median and the respective interquartile range boundaries; * - significant group difference according to ANCOVA adjusted for variability in age, sex and BMI with subsequent Bonferroni post-hoc test

Table III

Factorial analysis of the influence of variability in sex, age and BMI, as well as diagnosis of cataract on serum trace element and mineral levels

Element	Sex	Age	BMI	Cataract
Ca	0.289	0.578	0.609	0.321
Co	0.096	0.020 *	0.025 *	0.152
Cr	0.513	0.112	0.832	0.326
Cu	< 0.001 *	0.062	0.275	0.918
Fe	0.005 *	0.964	0.780	0.456
I	0.305	0.817	0.950	0.451
Li	0.030 *	0.840	0.143	0.177
Mg	0.089	0.025 *	0.651	0.902
Mn	0.095	0.972	0.845	0.026 *
Mo	0.021 *	0.579	0.003 *	0.860
Se	0.984	0.213	0.944	0.003 *
Sr	0.957	0.889	0.232	0.391
V	0.347	0.930	0.447	0.010 *
Zn	0.022 *	0.017 *	0.631	0.177

Data are expressed as p values of the factorial influence according to ANCOVA; * - the influence is significant at p < 0.05

The factorial analysis also demonstrated a significant impact of a diagnosis of cataracts on serum Mn, Se and V levels (Table III). Variability in the examinees' sex was associated with serum Cu, Fe, Li, Mo and Zn levels. In turn, age differences significantly impacted circulating Co, Mg and Zn concentrations. Finally, variability in BMI significantly affected the level of Co and Mo in the examinees' blood serum.

In contrast to serum, hair analysis demonstrated that only the hair Zn level in patients with cataracts was significantly 13% lower than that in eye disease-free controls (Table IV). Similarly to serum samples, patients with cataracts also had 25% lower hair Co content, although this difference was only nearly significant. No significant difference in other trace elements and minerals was observed between the study groups.

Table IV

Hair trace element and mineral content in subjects with and without age-related cataract

Element	Control	Cataract	P value
Ca	725.4 (389.2 - 1326.3)	512.5 (313.8 - 1564.8)	0.544
Co	0.012 (0.006 - 0.024)	0.009 (0.006 - 0.016)	0.062
Cr	0.205 (0.084 - 0.405)	0.164 (0.099 - 0.322)	0.348
Cu	12 (9.5 - 19.4)	11.8 (10 - 21.8)	0.736
Fe	13.7 (10.9 - 21.4)	14.7 (10.5 - 21.2)	0.962
I	0.51 (0.3 - 1.04)	0.409 (0.2 - 1.327)	0.886
Li	0.014 (0.007 - 0.028)	0.018 (0.01 - 0.031)	0.482
Mg	63.4 (32.7 - 118.5)	49.6 (29.3 - 133.2)	0.511
Mn	0.372 (0.269 - 0.637)	0.559 (0.26 - 0.736)	0.511
Mo	0.021 (0.017 - 0.027)	0.024 (0.015 - 0.029)	0.894
Se	0.44 (0.351 - 0.535)	0.407 (0.269 - 0.507)	0.264
Sr	2.07 (0.922 - 5.465)	2.05 (0.724 - 6.507)	0.940
V	0.023 (0.009 - 0.041)	0.018 (0.008 - 0.037)	0.580
Zn	194.7 (154.9 - 222.2)	168.6 (132.5 - 193.7)	0.005 *

Data are expressed as median and the respective interquartile range boundaries; * - significant group difference according to ANCOVA adjusted for variability in age, sex and BMI with subsequent Bonferroni post-hoc test

Generally, the results of factorial analysis corroborate group comparisons (Table V). Specifically, the diagnosis of cataracts significantly and nearly significantly impacted hair Zn and Co levels, respectively. At the same time, the examinees' variability in sex was associated with significant fluctuations in hair Ca, Mg, Mn, Mo and Sr levels. In turn, age and BMI significantly impacted hair Sr and Zn, respectively.

It is also notable that a significant correlation between hair and serum levels was observed for Cr (r = 0.268; p = 0.013), I (r = 0.298; p = 0.006), Li (r = 0.246; p = 0.044), Se (r = 0.271; p = 0.006) and Sr (r = 0.508; p = 0.001) in the general cohort of examinees.

To further specify the association between the levels of trace elements characterised by significant group differences in the examinees' hair and serum and cataracts and other diseases, multiple linear regression analysis was performed (Table VI).

Table V

Factorial analysis of the impact of sex, age, BMI and the presence of cataract on hair trace element and mineral levels

Element	Sex	Age	BMI	Cataract
Ca	0.003 *	0.768	0.900	0.549
Co	0.686	0.701	0.154	0.065
Cr	0.063	0.972	0.687	0.402
Cu	0.619	0.610	0.545	0.877
Fe	0.919	0.805	0.446	0.886
I	0.652	0.748	0.328	0.844
Li	0.891	0.715	0.061	0.463
Mg	0.001 *	0.375	0.649	0.626
Mn	0.043 *	0.883	0.280	0.518
Mo	0.002 *	0.807	0.429	0.578
Se	0.218	0.753	0.202	0.287
Sr	< 0.001 *	0.024 *	0.077	0.848
V	0.119	0.377	0.427	0.839
Zn	0.208	0.377	0.019 *	0.017 *

Data are expressed as p values of the factorial influence according to ANCOVA; * - the influence is significant at p < 0.05

Table IV

Multiple linear regression analysis of the association between serum and hair trace element levels with cataract after adjustment for anthropometric parameters and multiple comorbidities (only significant associations are provided)

	ICD-10	Serum Zn		Serum Se		Hair Zn		Hair Co		Serum Co	
		β	p	β	p	β	p	β	p	β	p
Sex	-	0.051	0.747	0.174	0.335	-0.063	0.693	-0.297	0.087	-0.141	0.417
Age	-	-0.268	0.115	-0.122	0.518	-0.402	0.015*	0.120	0.489	0.097	0.596
BMI	-	0.322	0.046*	0.041	0.822	-0.405	0.018*	-0.444	0.016*	-0.467	0.009*
Age-related cataract	H25	-0.768	0.003*	-0.576	0.044*	-0.172	0.482	-0.133	0.611	-0.138	0.609
Glaucoma	H40	0.106	0.477	0.134	0.426	-0.063	0.669	-0.337	0.036*	0.133	0.412
Disorders of refraction and accommodation	H52	0.515	0.005*	0.365	0.068	0.079	0.649	-0.144	0.440	-0.090	0.635
Type 2 diabetes mellitus	E11	-0.129	0.386	0.276	0.102	-0.008	0.958	0.430	0.007*	0.021	0.895
Varicose veins of lower extremities	I83	0.374	0.141	0.272	0.333	0.040	0.858	-0.473	0.055	0.587	0.036*
Hypotension	I95	0.381	0.023*	0.294	0.117	-0.103	0.535	-0.329	0.069	0.100	0.570
Angina pectoris	I20	-0.098	0.433	-0.116	0.410	0.329	0.007*	-0.047	0.710	-0.149	0.276
Phlebitis and thrombophlebitis	I80	-0.305	0.198	-0.280	0.286	-0.185	0.368	0.262	0.237	-0.623	0.018*
Chronic cholecystitis	K81.1	-0.143	0.258	-0.202	0.156	0.271	0.036*	0.048	0.726	0.038	0.778
Malaise and fatigue	R53	0.218	0.176	-0.099	0.585	-0.206	0.210	0.440	0.015*	-0.303	0.084

	ICD-10	Serum Zn		Serum Se		Hair Zn		Hair Co		Serum Co	
		β	p	β	p	β	p	β	p	β	p
Nail diseases	L60	-0.455	0.031*	-0.343	0.143	-0.527	0.011*	0.247	0.255	0.309	0.170
Xerosis cutis	L85.3	-0.026	0.864	0.173	0.316	-0.052	0.737	-0.066	0.692	-0.469	0.007*
Androgenic alopecia	L64	-0.078	0.542	-0.116	0.421	-0.271	0.040*	0.234	0.095	-0.073	0.599
Osteoarthritis, unspecified site	M19.9	0.401	0.041*	0.373	0.089	-0.242	0.216	-0.184	0.378	-0.141	0.499
Nervousness	R45.0	-0.096	0.497	-0.210	0.191	0.171	0.258	0.070	0.666	0.414	0.010*
Dizziness and giddiness	R42	-0.454	0.004*	-0.146	0.382	0.280	0.053	-0.017	0.914	0.154	0.342
Multiple R	-	0.810		0.745		0.758		0.714		0.771	
Multiple R^2	-	0.656		0.554		0.575		0.510		0.594	
Adjusted R^2	-	0.328		0.139		0.234		0.116		0.207	
P for a model	-	0.013*		0.174		0.039*		0.189		0.084	

Data are expressed as regression coefficient (β) and the respective p values; the association is significant at $p < 0.05$.

Specifically, after adjustment for anthropometric parameters and other diseases, hair Zn was not significantly associated with age-related cataracts (H25). Age, BMI and the presence of nail diseases (L60) and androgenetic alopecia (L64) were inversely associated with hair Zn content. In turn, angina pectoris (I20) and chronic cholecystitis (K81.1) were positively associated with hair Zn levels in the studied cohort.

In the adjusted multiple linear regression model, hair Co was also unrelated to the presence of cataracts, although it was inversely associated with BMI values and glaucoma diagnosis (H40). At the same time, hair Co was positively related to type 2 diabetes mellitus (E11) and malaise and fatigue (R53) in the examined subjects.

Similarly to hair Co, serum Co concentrations were found to be unrelated to the presence of cataracts. At the same time, BMI values, phlebitis and thrombophlebitis (I80) and xerosis cutis (L85.3) were inversely associated with serum Co levels. In contrast, the diagnosis of varicose veins of lower extremities (I83) and nervousness (R45.0) was characterised by a positive relationship with the studied parameter.

In agreement with group comparisons, serum Se levels were inversely associated with cataracts (H25) but not any other diseases present in the examinees. However, the overall regression model was not significantly associated with fluctuations in serum Se concentrations.

In the adjusted multiple linear regression model, serum Zn level was inversely associated with the presence of age-related cataracts, nail diseases (L60) and dizziness and giddiness (R42). At the same time, circulating Zn concentration was positively associated with BMI values, hypotension (I95) and unspecified arthrosis (M19.9).

In addition, serum V levels were not significantly associated with cataracts in the adjusted regression model (data not shown) while being positively related to age (β = 0.423; p = 0.039) and nail diseases (β = 0.578; p = 0.050). In contrast, unspecified osteoporosis was inversely associated with circulating V concentrations (β = -0.443; p = 0.048). Finally, serum Mn concentration was not significantly associated with cataracts or other eye diseases (data not shown). Only a significant association with the presence of seropositive rheumatoid arthritis was revealed (β = -0.399; p = 0.026).

The obtained data demonstrate that cataract patients are characterised by significantly lower hair Zn and serum Mn, Se, V and Zn levels compared to subjects without eye diseases. At the same time, after adjustment for other diseases diagnosed in the examinees, the inverse association with cataracts remained significant only for serum Se and Zn.

The revealed association of reduced blood and hair Zn levels with age-related cataracts provides further support for the potential role of Zn deficiency in the development of senile cataracts, although previous findings are rather contradictory. On the one hand, Zn deficiency assessed by plasma Zn levels was twofold more frequent in patients with cataract patients than non-cataract examinees [26]. At the same time, another study demonstrated that Zn deficiency was more profound in patients with diabetic cataracts than those with senile cataracts [27]. Although patients with senile cataracts were characterised by a significant decrease in plasma SOD and serum Zn levels with a concomitant increase in serum TBARS concentrations, in adjusted models, only SOD and TBARS, not Zn levels, were significantly associated with cataracts [28]. No significant group difference in blood Zn levels between patients with cataracts and healthy controls or association between aqueous humour Zn content and cataract severity was observed [29]. Furthermore, some studies demonstrated even higher blood Zn and Cu levels in patients suffering from cataracts, whereas only Mg was significantly lower than healthy controls [30].

In contrast to the existing epidemiological data, experimental studies provide evidence of the protective effects of Zn against cataract development [31]. Specifically, Zn and grape seed extract supplementation attenuated senile cataract development in Wistar rats by inhibiting oxidative stress [32]. In rats exposed to total cranium radiotherapy, Zn supplementation significantly reduced TBARS, Fe and Ca levels in the lens while increasing SOD and GPx activity [33]. Furthermore, in radiation-exposed rats, Zn could also increase glutathione reductase and glutathione-S-transferase, as well as total superoxide scavenging activity while reducing lens xanthine oxidase activity [34]. It is suggested that exogenous Zn induces stoichiometric changes in metallothionein molecule modulates Zn-MT redox system antioxidant capacity [35]. Zn also up-regulated MrsB1 activity, reduced oxidative stress and inhibited protein carbonylation in human lens epithelial cells under normal and prooxidant conditions [36]. In addition to reducing oxidative stress, protective effects of Zn in human lens epithelial (HLE B-3) cells were associated with attenuation of mitochondrial dysfunction and improvement of Ca^{2+} homeostasis [37].

The observed inverse association between serum Se level and cataracts generally corresponds to the essential role of Se in eye physiology [11]. At the same time, selenium overload is known to result in the development of cataracts in experimental animals [38] through ROS overproduction and endoplasmic reticulum stress *via* inhibition of Nrf2/Keap1 signalling [39]. However, no significant increase in the prevalence of cataracts in the high-Se area of China was observed [40]. In turn, serum and aqueous humour selenium levels were lower in cataract patients than in healthy controls [41]. Low serum Se levels were associated with a higher prevalence of age-related, nuclear and cortical cataracts, whereas no relationship between GPX-1 and GPX-4 single nucleotide polymorphisms was observed and cataracts observed [15]. Correspondingly, the results from NHANES demonstrate a significant inverse association between Se intake and the incidence of cataracts, specifically in women aged 65 - 74 years, indicating the potential protective effect of Se supplementation against senile cataract development [42]. Furthermore, it has also been demonstrated that Se supplementation significantly reduced the risk of cataracts in The Selenium and Vitamin E Cancer Prevention Trial (SELECT) Eye Endpoints Study [43]. In contrast, high plasma glutathione peroxidase activity is associated with an increase in the prevalence of nuclear and mixed cataracts, especially cortical cataracts in the POLA study [44].

The potential protective effects of Se against cataracts are expected to be mediated by the antioxidant activity of certain selenoproteins. Specifically, both ebselen and selenite were shown to prevent D-galactose-induced cataracts by reducing oxidative stress in rat lens through up-regulation of selenoprotein R, 15kD selenoprotein, SOD1, catalase and β-crystallin expression, while reducing that of aldose reductase and glucose-regulated protein 78 [45]. These findings corroborate earlier observations of cataract development in the early age of 15-kDa selenoprotein knockout mice [46]. Glutathione peroxidase-1 deficiency is associated with oxidative stress in the lens, membrane damage and cataract formation [47]. Notably, ebselen, an organoselenium compound, attenuated the progression of selenite-induced cataracts by inhibiting oxidative stress, evidenced by reduced MDA and nitrite levels in the lens [48]. These findings demonstrate that adequate supplementation with Se may be considered protective against cataract formation. In contrast, its deficiency and excess may be considered a risk factor for cataract development [49].

Although no previous studies demonstrated an association between the internal dose of V and cataracts, V has protective effects against cataractogenesis in diabetic rat models [50] through improving carbohydrate metabolism and lens antioxidant status [51].

We have also revealed a significant decrease in serum Mn levels in cataract patients, although this association was not significant after multiple adjustments. Earlier studies demonstrate that the level of Mn in the lens was significantly reduced in patients with senile cataracts compared to the normal lenses [52, 53]. At the same time, no significant group difference in blood Mn was observed between cataract cases and controls, while aqueous humour Mn concentration was unrelated to cataract severity [29]. Agreeing with the present findings, Mn intake was inversely associated with reduced risk of nuclear cataracts [54]. At the same time, in a cohort exposed to multiple metals, urinary Mn concentrations were associated with age-related cataracts in an unadjusted model [55]. Therefore, the role of Mn in cataract pathogenesis is yet to be estimated.

The main strength of the present study is a detailed analysis of case histories and adjustment of the revealed associations not only for comorbid eye disease but also for other diseases present in the examinees. At the same time, the present study has certain limitations. First, a higher number of examinees would be beneficial for a more detailed assessment of the relationship between age-related cataracts and trace element and mineral levels. Second, the study would benefit from additional evaluation of disease severity and analysis of trace element and mineral levels in eye tissues, especially cataractous lenses.

Conclusions

The obtained data demonstrate that low serum zinc, especially selenium levels, are independently associated with an increased risk of cataracts. Therefore, improving zinc and selenium status may protect against age-dependent cataract development, especially in advanced-age subjects. However, further studies are required to investigate additional biomarkers of Se and Zn metabolism in subjects with different cataract severity and unravel the underlying molecular mechanisms.

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Conflict of interest

The authors declare no conflict of interest.

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