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RESEARCH ARTICLE

Correlation Study of Serum Zinc Concentration and Retina Layer Thickness in Hypertensive Patients

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Objective: The aim of our study was to evaluate whether blood serum zinc concentration correlates with the thickness of human retina layers, in hypertensive patients with microvascular damage. **Methods**: Retinas of elderly patients with arterial hypertension and microvascular damage were imaged using a swept-source ocular coherence tomography from Topcon. Automatic retinal segmentation was applied on a 6mm X 6mm scan protocol and average thickness for 5 examined layers was used for statistical analysis. Serum zinc concentration was measured using the Zinc Assay kit from Sentinel Diagnostics in a spectrophotometric method. **Results**: The average age of the twenty-three enrolled patients was 70 years, varying between 62 and 76. The mean zinc value was 9.9 µmol/l ±1.62 (SD). All five examined layers of the retina presented inverse correlation with serum zinc concentration. The complex including the inner plexiform layer and ganglion cell layer indicated the Spearman's (rho) correlation coefficient -0.42 and a significance level of p=0.04. Patients in high-Zn group (≥ 9.87 µmol/l) had thinner macular retina layers, most importantly in the inner-plexiform layer-ganglion cell layer complex (p=0.006). **Conclusions**: Our study has found that serum zinc concentration is inversely correlated with the thickness of retina layers with statistical relevancy in the inner plexiform layer – ganglion cell layer complex. This finding emerges experimental studies in order to elucidate its clinical significance and to evaluate whether the fine architecture of the inner retina has the potential to benefit from oral zinc supplementation through modulating serum levels of zinc in patients with microvascular-damaging diseases.

Keywords: zinc, retina, arterial hypertension, microvascular disease

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Introduction

Zinc is an essential modulator in human physiology and extensive research was conducted in order to discover its localization in specific tissues. Measurements of zinc in humans found the highest concentration in the retina-choroid complex, being approximately 20 times more abundant here than in most organs [1]. Within the retina, the distribution of zinc shows layer specificity. In the rat retina, zinc was found inside Golgi apparatus in the pigment epithelium layer (RPE), in the inner segment of photoreceptors, through the inner nuclear layer (INL) and inside neuronal processes of the outer and inner plexiform layers [2].

Zinc localization and relevance at the outer edge of the retina was previously characterized including pathologic conditions of human eyes such as age-related macular degeneration (AMD) [3,4]. Recently, new research brought to our attention the inner retina's inner plexiform layer (IPL) where high amount of mobile zinc is released from amacrine cell processes after optic nerve injury [5]. Also, tubulin polymerization promoting protein (TPPP) – a microtubule stabilizing, and zinc binding protein was exclusively localized in specific amacrine cells and their processes in the IPL [6].

Additional particularity of the inner retina is its extensive network of blood capillaries. The greatest vascular density in the retina lays at the inner and outer edge of the IPL with interconnections crossing the layer [7]. Furthermore, the presence of an intersublaminar vascular plexus within the IPL was identified [8]. Through this exclusive vascular network, the inner retina is connected with a direct supply of zinc through the blood stream. The zinc pool from the blood interacts with the endothelium of retinal capillaries. Endothelial dysfunction has been linked to zinc homeostasis, showing a protective effect of zinc on endothelium [9, 10].

Reviewing relevant reports, the inner retina and especially the IPL appears to be a membrane impregnated in zinc and interacting cytoskeletal related molecules, and enmeshed by the zinc pool of the blood. Furthermore, zinc has the capacity to act on the constituents of microvasculature of this layer. Consequently, we hypothesized that the inner retina and especially the IPL could be susceptible to changes based on available amount of zinc from the blood pool. If the changes are great enough, they could be detectable with available imaging method such as the cross-sectional scan of an ocular coherence tomography (OCT). The aim of our study was to evaluate whether the serum zinc concentration correlates with the thickness of layers of the retina, especially within the inner retina, in patients with arterial hypertension and microvascular damage. We set up the following objectives: to measure the thickness of several retina layers and the serum zinc level, and to perform statistical analysis between the obtained numerical data.

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Methods

We performed a cross-sectional study enrolling elderly patients (above 60 years of age) with microvascular disease secondary to arterial hypertension, admitted in the Cardiovascular Rehabilitation Clinic of Targu Mures. The timeframe for enrolment encompassed consecutive 12 months beginning with October 2016. Fundus photography of the macular and optic nerve head regions were acquired using the Carl Zeiss Visucam 500 for evaluating the microvascular damage to the retina. The imaging was performed after pupillary dilatation with one drop/eye of 10 mg/ml Tropicamide in both eyes, except in cases in which cataract allowed only one eye examination. After Tropicamide instillation, 30 minutes repose was aimed for obtaining optimal pupillary dilatation. Microvascular damage was defined based on the following signs of retinopathy: constricted or tortuous arterioles, vascular nicking, silver or copper wire sign, hemorrhage, hard exudate, cotton wool spot, microaneurism, Elschnig spot and Siegrist streak. The presence of epiretinal membrane and tractional retinal detachment were not considered exclusion criteria, as they are related to microvascular damage. Data from the most affected retina or in the only available retina of each patient was further analyzed.

Patients with history of neurological disorders such as dementia or cognitive impairment and eye diseases including glaucoma or elevated intraocular pressure, optic neuropathy and previous vitreoretinal surgery were excluded from the study.

All included patient underwent macular OCT scans applying the 6mm X 6mm protocol using a swept-source Dri Triton OCT by Topcon. We targeted obtaining the highest quality images with dilated pupils, and no OCT scan with image quality beneath 50 (out of 100) was included. The average thickness corresponding to this area was automatically calculated for multiple layers after applying segmentation through the built-in software of the OCT for the layers presented in Figure 1.

The serum concentration of zinc was measured using the automated photometric analyzer - Konelab 20 XTi by Thermo Fisher Scientific and the Zinc Assay kit from Sentinel Diagnostics (product code 17640H). Fasting serum samples were used for zinc measurement. Venous blood was collected in 6 ml vacutainers without additives. The blood was left to clot for 30 minutes, after centrifugation (10 minutes at 5000 rpm) serum was separated from the cellular fraction within one hour. Serum samples (1.5 ml) were transferred to metal-free polypropylene vials, avoiding transfer of cellular elements of the blood. Hemolyzed blood samples, icteric and lipemic samples were excluded from the study. Serum samples were stored frozen at -20 ^{o}C for 3-4 months before serum zinc measurement. Calibration of the analyzer was performed with calibrators included in the commercial kit. Controls were tested before measurement of serum samples of the patients; control results were included in the interval provided by the manufacturer. Results obtained from the patient's serum samples showed Gaussian distribution. Measurement uncertainty was in the range indicated by the manufacturer.

Based on the measured serum zinc concentrations median value, patients were later divided into higher- and lower-zinc groups in order to assess correlations with retina layers thickness.

We used MeDCalc® version 18.6 for statistical analysis. Spearman (rho) test was applied for calculating correlations using a 95% confidence interval, and Mann-Whitney U test for group comparisons.

The study obtained ethical approval from the Ethical Committee of the County Emergency Clinical Hospital of Targu Mures. The scope and procedure of the study was explained to participants and enrolment was on voluntary basis. The study was conducted in accordance with the Declaration of Helsinki for medical research involving human subjects.

Results

Twenty-three patients were included in the study, 13 women and 10 men, with a mean age of 70 years, varying between 62 and 76. Therefore, 23 eyes from 23 patients were selected for macular thickness measurements.

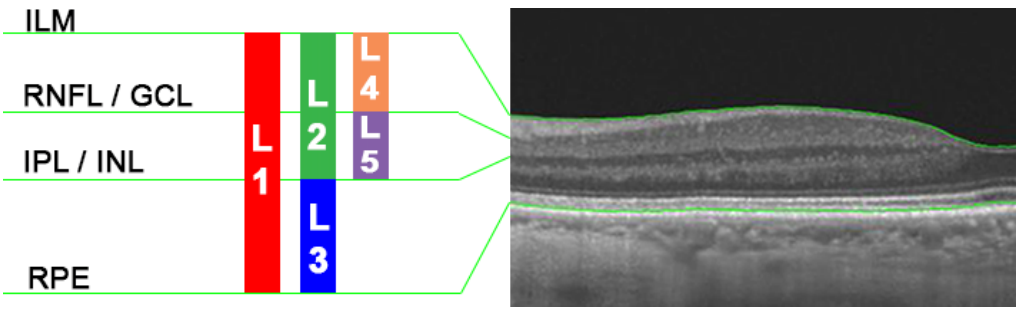

Fig. 1. Schematic representation of the segmentation in the macular region. The green horizontal lines represent the level of segmentation through the retina, corresponding to the retina layers visualized on the OCT scan. The L1-L5 color-bars represent the thickness of layers considered for correlation assessments. Segmentation lines were automatically placed at the inner limiting membrane (ILM) and retinal pigment epithelium (RPE) for measuring total retinal thickness. The limit between the retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL), and the limit between the inner plexiform layer (IPL) and inner nuclear layer (INL) were also marked in order to calculate average thicknesses of resulting layers. This segmentation distinguished 5 distinct layers (from L1 to L5). The relation between the segmentation layers obtained on the OCT and anatomical layers are: L1 (total retina thickness), L2 (RNFL, GCL, IPL), L3 (INL, OPL – outer plexiform layer, PhRL – photoreceptor layer, RPE), L4 (RNFL) and L5 (IPL, GCL).

The mean zinc value was 9.9 μmol/l ±1.62 (SD). The reference interval for physiological values provided by the manufacturer was 10.4-16.4 μmol/l.

The main result on correlations between serum zinc concentration and thickness of retina layers in macular area are presented in Table I.

As no limit at which serum zinc concentration could influence retinal layer thickness in healthy retina or in patient with microvascular disease was previously published, in order to further investigate the identified inverse correlation, we performed a follow-up analysis. Patients were divided into higher- and lower-Zn groups considering the median value of serum zinc (9,87 μmol/l). Thereby, retina layer thicknesses of 11 patients with zinc values lower than this limit were compared to retinas of 12 patients with values ≥9.87 μmol/l. Once again, the layers of the inner retina were significantly thicker in patients with lower concentration of zinc in blood (Table II).

Discussion

The inclusion criteria applied to the study population was based upon our ophthalmology cases on daily bases. In our experience, elderly patients are more likely to receive prescription of oral zinc supplementation. Since the Age-Related Eye Disease Studies (AREDS) which have proven the benefit of zinc supplements in slowing the progression of Age-Related Macular Degeneration [11,12], these are regularly prescribed by ophthalmologists. The benefit of zinc on several aspects of retinal health is well known by clinicians [13]. Additionally, our patients frequently present associated microvascular damaging diseases such as chronic arterial hypertension or diabetes mellitus type 2. Studies have found that two thirds of patients above 65 years old suffer from arterial hypertension [14]. Thus, we considered adequate to answer the hypothesis in a cohort with characteristics of grater clinical frequency. However, in our study, we strictly targeted the serum zinc concentration with no evaluation of alimentary zinc intake. Exclusion criteria were based on knowledge about diseases of neurological origin and glaucoma, or conditions post-surgery that separately had the potential of modifying retinal layer thickness [15-18].

The finding that lower concentration of zinc in blood serum tend to correlate with thicker retina layers and vice versa may have clinical impact when suggesting zinc supplements to our patients with retinal microvascular disease. However, our data only suggests the necessity of further studies in this direction. For this reason, a disadvantage of this study is the incapacity of identifying a causal effect of serum zinc on the retinal layers. Thus, we are unable to evaluate whether oral zinc supplementation could be beneficial for the inner retina in patients with microvascular-damaging diseases. However, this study pointed out an interesting topic for future investigations with potential clinical outcome through modulating the neuronal architecture in the inner retina via zinc supplements. Until then, the choroid-Bruch's membrane-RPE complex in age-related macular degeneration remains the only target for ophthalmologists recommending oral zinc supplementation. A second limitation of the study is the small number of patients enrolled. We aimed to overcome this by analyzing high quality images, revising the quality of automated segmentation and carefully selecting the patients based on previously presented criteria.

Conclusion

This study involving elderly patients with microvascular-damaging disease has found that serum zinc concentration is inversely correlated with the thickness of human retina layers with statistical relevancy in the inner plexiform layer – ganglion cell layer complex. Furthermore, the patients with lower concentration of zinc in blood serum tend to have thicker retina layers and vice versa. This finding emerges experimental studies in order to understand its clinical importance and to evaluate whether the fine architecture of the inner retina has the potential to benefit from oral zinc supplementation through modulating serum zinc concentration.

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Table I. Retina layer thickness and correlation with serum zinc concentration. Spearman's (rho) indicates direction and strength of correlations. Highlighted in bold - marks statistically significant inverse correlation between zinc and corresponding layer.

Segmentation	Spearman's (rho)	Significance level (p)
L1	-0.35	0.09
L2	-0.33	0.11
L3	-0.22	0.29
L4	-0.26	0.22
L5	**-0.42**	**0.04**

Table II. Comparison of thickness of corresponding retina layers according to lower or higher serum zinc concentration. It is remarkable that in each case there is a thicker layer when lower zinc concentration is present and vice versa. Highlighted in bold - marks statistically significant differences.

Segmentation	Zn level <9.87μmol/l (mean thickness ±SD)	Zn level ≥9.87μmol/l (mean thickness ±SD)	Significance level (p)
L1	275.94 ± 8.62	265.94 ± 14.35	0,08
L2	**101.94 ± 3.83**	**95.02 ± 8.5**	**0,02**
L3	173.99 ± 10.83	170.92 ± 8.51	0,56
L4	28.48 ± 1.34	27.83 ± 4.02	0,2
L5	**73.4 ± 3.03**	**67.20 ± 5.66**	**0,006**

Authors' contribution

RGT (Conceptualization; Formal analysis; Investigation; Data curation; Writing - original draft; Writing - review & editing)
ENN (Investigation; Formal analysis; Data curation; Methodology; Validation; Writing - original draft; Writing - review &editing)
ZP (Investigation; Methodology; Data curation)
KUH (Conceptualization, Validation; Writing - original draft)
BB (Investigation; Methodology; Data curation)
AB (Conceptualization; Supervision; Writing - review & editing)
MGS (Conceptualization; Supervision; Funding acquisition; Writing - review & editing)

Conflict of interest

The authors declare no conflict of interest regarding this study.

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