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Antioxidant and free radicals species in the aqueous humor of patients with age-related cataract

Антиоксиданти и слободни радикали у очној водици болесника са сенилном катарактом

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SUMMARY

Introduction/Objective Age-related cataract is a significant cause of visual impairment worldwide. Oxidative damage and the effects of free radical species are considered important in the etiopathogenesis of cataracts.

The aim of this study was to evaluate the antioxidative capacity and oxidative stress in the aqueous humor according to age and cataracts maturity.

Methods Clinical and biochemical researches were carried out in 55 patients with age-related cataract. According to the cataract maturity patients were classified into incipient (cortical-group C, 18 pts, nuclear-group N, 20 pts) and mature (group M, 17 pts). In order to evaluate the impact of age patients within each group were divided in: Group I (65–69 years) and Group II (70 ≥ years).

The antioxidant activity of aqueous humor was measured by the reduction power (RP) method and the activity of glutathione peroxidase (GPx) spectrophotometrically. Changes in the concentrations of hydroxyl and ascorbyl radicals were detected by electron spin resonance spectroscopy.

Results Both RP and GPx activitiy were significantly ($p < 0.001$) reduced in group N compared to group C and in group M compared to group N. Concentrations of hydroxyl (29.45 ± 1.01% in group C, 38.12 ± 1.29% in group N and 74.14 ± 2.52% in group M) and ascorbyl radicals (26.12 ± 0.89% in group C, 41.15 ± 1.39% in group N and 83.56 ± 2.84% in group M) increased significantly ($p < 0.05$) with progression of age-related cataract. Significant negative correlation ($r = -0.759$, $p < 0.05$) was determined between concentrations of hydroxyl radicals and content of GPx.

Conclusion Our research proved that the level of oxidative stress in the aqueous humor is significantly affected during aging and cataract progression, The obtained data support the hypothesis that during aging, depending on the maturity of the cataract, the antioxidant capacity in the aqueous humor decreases due to an increase in the concentration of reactive hydroxyl radicals.

Keywords: cataract; antioxidant enzyme; hydroxyl radical; ascorbyl radical

САЖЕТАК

Увод/Циљ Сенилна катаракта је један од најчешћих узрока оштећења вида широм света. Оксидативна оштећења и слободни радикали значајно утичу на етиопатогенезу катаракте. Циљ овог истраживања је био одређивање антиоксидативног капацитета и оксидативниог стреса у очној водици пацијената у односу на старосну доб и зрелост катаракте .

Методе Клиничка и биохемијска испитивања су спроведена код 55 пацијената са сенилном катарактом. Према степену зрелости катаракте, сви пацијенти су подељени у групе: инципиентна (кортикална група *C*, 18 болесника, нуклеарна група *N*, 20 болесника) и зрела (група *M*, 17 болесника) катаракта. У доносу на старосну доб формиране су две групе: Група I (65–69 год.) и Група II (70 ≥ год.). Антиоксидативна активност у очној водици је мерена методом редукционе способности (*RP*) и активности ензима глутатион пероксидазе (*GPx*). Промене концентрација хидроксил и аскорбил радикала детектоване су електрон спин резонантном спектроскопијом.

Резултати *RP* и *GPx* активност су статистички значајно ($p < 0,001$) смањене у групи *N* у поређењу са групом *C* и у групи *M* у поређењу са групом *N*. Концентрације хидроксил (29,45 ± 1,01% у *C*, 38,12 ± 1,29 % у *N* и 74,14 ± 2,52% у групи *M*) и аскорбил радикала (26,12 ± 0,89% у *C*, 41,15 ± 1,39% у *N* и 83,56 ± 2,84% у *M*) значајно су порасле ($p < 0,05$) са прогресијом катаракте и старосном доби болесника. Утврђена је значајна негативна корелација ($r = -0,759$, $p < 0,05$) између концентрације хидроксил радикала и садржаја *GPx*.

Закључак Наше истраживање је доказало да на ниво оксидативног стреса у очној водици значајно утиче, поред старости, и степен зрелости катаракте. Добијени подаци подржавају хипотезу да се током старења, у зависности од зрелости катаракте у очној водици, антиоксидативни капацитет смањује услед повећања концентрације реактивних хидроксил радикала.

Кључне речи: катаракта; антиоксидативни ензим; хидроксил радикал; аскорбил радикал

INTRODUCTION

Age-related cataract is a common cause of visual impairment which can significantly reduce patient's quality of life. As the world's population ages, an increase in the number of patients is expected. Knowing these facts, it is not surprising that there is large number of researches aimed at determining the cause and prevention of this disease [1]. The pathophysiology of age-related cataract is complex and still not fully understood. Several risk factors such as diabetes, malnutrition, diarrhea, poverty, sunlight, smoking, hypertension, renal failure are associated with cataract formation [2]. Various studies have gradually confirmed that reactive oxidative species (ROS) play the most important role in the etiology of cataract formation. Opacification of the lens may be initiated by photochemically or non-photochemically oxidative damage [2]. The present hypothesis considers oxidative stress as an important factor which can damage the crystalline proteins, lipids, polysaccharides and nucleic acids during cataractogenesis [3].

By the nature of their functioning all aerobic organisms are continuously exposed to oxidants, such as free radicals (superoxide anion, hydroxyl, alkyl, peroxyl) and non-radical species (hydrogen-peroxide, ozone, singlet oxygen, organic peroxides). ROS levels are normally controlled by intracellular antioxidant defense mechanisms that include endogenous antioxidants such as enzyme systems [superoxide dismutase, catalase, glutathione peroxidase (GPx)], uric acid, bilirubin, glutathione, coenzyme Q10. Also, the exogenous antioxidants which include vitamin C, vitamin E, carotenoids, polyphenolic compounds participate in the stabilization and transformation of ROS in the secondary level of protection [4]. Unfortunately, with aging oxidative damage increases, antioxidant capacity decreases in the lens and in the aqueous humor (AH) and the efficiency of reparative systems become impaired. Such an imbalance in the organism is called oxidative stress, which is the cause or accompanying factor in the pathology of many diseases [5].

The secretion of AH and the regulation of its conventional and non-conventional pathway are physiologically important processes for normal eye function and their antioxidant capacity reflects the degree of oxidative stress in the surrounding tissues [6]. Understanding the mechanisms of cataractogenesis should bring a better therapy.

Conditions leading the excess hydrogen-peroxide (H_2O_2) in AH may precipitate oxidative damage and cataract formation. The oxidizing agent, H_2O_2 is present in AH at concentration of approximately 20–30 μmol and it is reported to be raised (up to 660 μmol) in patient with cataract. Higher than normal levels of H_2O_2 production in the lens and/or AH could be via intraventricular, autoxidation of ascorbic acid, GSH and 3-hydroxykynurenine. Protein modifications linked with cataract could be the result of a reaction of crystalline lens, with the hydroxyl radicals, which derive from H_2O_2 through the transition-metal ion catalyzed Fenton reactions [7].

Several studies point out the possibility that one of the main functions of high concentration of ascorbic acid (vitamin C) in AH is to protect the lens and other surrounding tissues against the oxidative stress induced by free radicals. In addition, large concentrations of ascorbic acid in the AH appear to provide significant protection against oxidative insult, and this possibly explains the occurrence of a high concentration of ascorbic acid in the AH [8]. Deprotonated form of ascorbic acid (ascorbate) forms covalent bonds with the crystalline lens, which reduces protein solubility [9]. But in the presence of ROS, particularly H_2O_2 or hydroxyl radicals, in the first and second one-oxidative reactions of ascorbic acid, ascorbyl radical and then dehydroascorbic acid it could be formed [10]. These reactions occur during increased oxidative stress when the mechanism for maintaining the reduced form of ascorbic acid is compressed.

The difficulty in studying the role of free radicals in human cataract formation is the inability to measure directly these reactive species in the lens or AH *in vivo*. Electron spin

resonance (ESR) spectroscopy provides a unique method to examine directly free radicals and it gives information about the concentration and structure of the radical centre's surroundings. Owing to the unpaired electron in the outer orbital, free radicals are paramagnetic species and when present in sufficient quantity, are detectable and measurable by ESR spectroscopy [11, 12]. Kinetic measurements and analysis the change in parameters of ESR spectra (line shape, linewidth, line intensity and *g*-factor) reflect important data about the reactions of free radicals in the AH due to the fact the epithelial surface of the lens is in contact with the aqueous fluid.

The purpose of this paper is to analyze parameters of oxidative stress by testing the redox power (RP) and enzyme antioxidant power and formations of ascorbyl and hydroxyl radicals in the AH of patients with different age and maturity of age-related cataract.

METHODS

Chemicals and reagents

All chemicals and solvents were of the highest analytical grade.5,5-dimethyl-1-pyroline-N-oxide (DMPO), Trolox, potassium ferricyanide and trichloroacetic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). Ferric chloride was obtained from J.T. Baker (Deventer, Holland) and sodium nitrite from LACH-NER (Brno, Czech Republic). Total GPx assay kit was from Helvetica Health Care Sàrl, Geneva, Switzerland.

Patients

Clinical and biochemical researches were carried out in 55 patients (P1-P55) with age-related cataract. According to the cataract maturity patients were classified into incipient (cortical-group C, 18 pts, nuclear-group N, 20 pts) and mature (group M, 17 pts). In order to evaluate the impact of age patients within each group were divided into: Group I (65-69 years) and Group II (70 years).

Patients with other ophthalmic (glaucoma, uveitis, retinal diseases, ect.) and systemic (diabetes, hyperlipemia, immunological ect.) diseases that might have influence on oxidative stress were excluded.

Sample collection

The samples of the AH for analysis were taken immediately before the start of surgical procedure (phacoemulsification with intraocular lens implantation). All operations were performed according to the principles of sepsis and antisepsis. Through lateral limbal paracentesis from the space of the anterior chamber 0.15-0.20 ml, of AH was aspirated using a Gliss Wells cannula of 20 G. The amount of AH taken was compensated with isotonic Ringer-lactate solution and the surgical procedure was continued as usual.

Biochemical analysis of reduction power

RP was determined by the method of adapted for a 96-well microtiter plate [13]. Eppendorf tubes contained 75 μl of sample solution or 75 μl of extractant (blank test), 75 μl of Na-phosphate buffer, pH 6,6, and 75 μl of 1% potassium ferricyanide. Incubation was performed at 50˚C and then 75 μl of 10% trichloroacetic acid was added. After centrifugation, 50 μl of distilled water and 10 μl of 0.1% ferric chloride were added to 50 μl of carefully separated supernatant. The absorbance of the samples was measured at a wavelength of 700 nm. A calibration curve was constructed with Trolox, and the results were expressed as mmol Trolox equivalents per ml of sample (mmolTE/ml).

Glutathione peroxidase activity assay

The activity of GPx was determined spectrophotometrically (UV-1800 spectrophotometer, Schimadzu, Kyoto, Japan). Total GPx assay kit provides a method of

quantifying the activity of GPx (U/ml) [14]. The oxidation of NADPH to $NADP^+$ is monitored by a decrease in absorbance at A340.

ESR detection of reactive hydroxyl radicals

Based on the fact that hydroxyl radicals formed in Fenton's model system have a short lifetime (<< 1ms) and low concentration ($<10^{-7}$M), ESR spectroscopy is combined with the "spin-trapping" method. This technique involves the addition-type reaction of a short-lived radical with a paramagnetic compound (spin-trap) to form a long-lived free radical product (spin-adduct), which can then be studied using ESR. In this work, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was used as a spin trap, and the concentration of the resulting DMPO-OH is equivalent to the concentration of hydroxyl radicals [12]. The system consisting of: 500 μmol H_2O_2, 75 μmol $FeCl_2$, 100 mmol DMPO (control sample). Data refer to the ESR signal intensity of DMPO-OH detected in the control sample defined as 1 (100%).

The influence of AH on the amounts of hydroxyl radicals trapped by DMPO was studied by adding 20 μl AH to the control system. ESR spectra were recorded with the following spectrometer settings: modulation amplitude 0.512 G, x-band frequency 9.64 GHz, receiver gain 1×10^4, center field 3440.00 G, sweep width 100.00 G, time constant 81.92 ms, conversion time 163.84 ms, power 20 mW.

The relative intensity of hydroxyl radicals ($RI_{\bullet OH}$) value of the samples was defined as:

$$RI_{\bullet OH} = 100 \times (h_x - h_0) / h_0 \ [\%]$$

where h_0 and h_x are the hight of the second peak in the ESR spectrum of DMPO-OH spin-adduct of the control sample and the probe (control sample with AH), respectively.

ESR detection of ascorbyl radicals

Ascorbyl radicals in AH were directly measured by ESR spectrometer with the same

spectrometer settings adjusted for the determination of hydroxyl radicals.

Statistical analysis

All analysis were run in triplicate and were expressed as means±standard deviation (SD). Statistical analyses were carried out using Origin Pro 8.0 software. Significant differences were calculated by ANOVA and Tukey's test. ($p < 0.05$).

We certify that institutional regulations concerning the ethical use of human volunteers were followed during this research. This study was approved by the Human Subjects Committee of the University of Novi Sad (00-209, November 26th, 2021) and adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants.

RESULTS

AH samples were obtained from 55 patients (P1-P55). The mean age in Group I was 67.41 ± 2.89 and 78.23 ± 3.42 in group II. There was no significant difference in gender and cataract type between Group I (cortical 9 pts, nuclear 10 pts and mature 8 pts) and Group II (cortical 9 pts, nuclear 10 pts and mature 9 pts).

Among our patients, a significantly ($p<0.001$) higher concentration of RP was measured in AH of younger patients (Group I) with incipient cataract (C group 1.81± 0.07 mmolTE/ml; N group 0.76±0.04 mmolTE/ml) compared to the mature (0.32±0.08 mmolTE/ml) cataract. In elderly patients (Group II) the antioxidant status of AH decreases, what is the consequence that RP value also decreases in patients with incipient (C group 1.02± 0.04 mmolTE/ml; N group 0.66±0.03 mmolTE/ml) and especially in those with mature cataract (0.15±0.01 mmolTE/ml).

Analyzing the GPx activity in AH of patients with cataract, our results showed that the GPx activitiy were significantly reduced in the AH of patients with nuclear as compared to the cortical cataract. Testing the difference, depending on the maturity degree of the cataract, a

significantly lower activity of GPx were measured in the group of patients with mature cataract in relation to the nuclear cataract group. The mean value of GPx in AH of patients in the Group II significant decreased on 2.81±0.13 U/ml in C, 1.72 ± 0.06 U/ml in N and 0.98 ± 0.03 U/ml in M group, respectively, when compared with that of the patients from Grop I in all cataract maturity (Table 1).

Mean values of the percentage increase in the intensity of the ESR signal of DMPO-OH spin-adduct and ascorbyl radical, which indicates an increase in the concentration of hydroxyl and ascorbyl compared to the control sample, are shown in Table 2.

Testing the differences, depending on the cataract maturity, a significantly higher concentration of hydroxyl radicals was found in AH with the patients with mature cataract, and the production of hydroxyl radicals increases during the process of aging. The hydroxyl radical concentrations in AH of patients in the 65–69-year age group increased for 29.45 ± 1.01 % in C, 38.12. ± 1.29 % in N and 74.14 ± 2.52 % in M group, respectively. In patients from ≥70 group the intensity of ESR signal increase is higher (36.22 ± 1.82% in C, 48.72 ± 1.71 % in N and 85.50 ± 1.94% in M group), respectively.

In all 55 cases a typical ESR spectrum of DMPO-OH spin-adduct, with four lines of relative intensities 1:2:2:1 and hyperfine splitting constant $a_N = a_H = 14.9$ G was observed. Increase in the relative intensity (RI) of ESR signal of DMPO-OH spin-adduct expressed in percentages relative to the control sample. The examples of ESR spectra obtained in the AH of patient P15 with cortical cataract are presented at Figure 1A. Figure 1B shows the ESR signal of DMPO-OH spin-adduct detected in the AH of a patient P31 diagnosed with nuclear cataract. The highest ESR signal intensity of the DMPO-OH signal was registered in patient P50 with mature cataract (Figure 1C). The mean values of the percentage increase in the ESR signal of DMPO-OH spin adducts are summarized in Figure 2.

The ascorbyl radical concentrations in AH in the 65–69-year age group increased for

26.12±0.89% in C, 41.15±1.39% in N and 83.56±2.84% in M group, respectively. In the group of patients over 70 years old, the increase in the intensity of the ESR signal of ascorbyl radicals is much higher (36.20 ± 1.82 % in C, 58.75 ± 2.06 % in N and 90.65 ± 2.96 % in M group), respectively (Table 2).

The free radicals obtained directly in AH of patients with cataract were characterized by ESR spectroscopy as a simple doublet showing coupling constants of aH =1.84 G. According to literature data this can be assigned to an ascorbyl radical [12]. The unpaired electron in the structure of the ascorbyl radical is located in the π-system that includes the tri-carbonyl group of ascorbates. Thermodynamically, it is relatively unreactive with a one-electron reduction potential of only +282 mV. The examples of typical ESR spectra obtained in the AH of patients with cortical, nuclear and mature cataract, are presented at Figure 3A, 3B and Figure 3C. The mean values of the percentage increase in the ESR signal of ascorbyl radicals are summarized in Figure 4.

The concentrations of hydroxyl radicals were significantly positively correlated to the concentrations of ascorbyl radicals ($r = 0.8355$; $p < 0.05$) (Figure 5) in AH of patients with age-related cataract while the activity of GPx significantly negatively correlated with the increase in the concentration of hydroxyl radicals ($r = -0.7590$; $p < 0.05$).

DISSCUSION

This study showed, that the level of oxidants and antioxidants present in AH, in addition to the age of the patients, is also significantly influenced by the maturity of the cataract. We emphasize on the GPx activity changes seen in the AH of individuals with incipient cataract (cortical group C, 18 pts; nuclear group N, 20 pts) and mature cataract (group M, 17 pts). Our results indicate a significant reduction of GPx concentrations in patients with nuclear cataract compared to the patients with cortical cataract. (Table 1).

Chronic lens exposure to molecular oxygen conditions the decline in the status of antioxidant enzymes, which conditions the pathogenesis of nuclear cataract [15]. This fact is responsible for increased oxidative stress that causes protein damage in the lens core, protein aggregation, light scattering and loss of lens transparency. Compounds with reducing ability, as electron donors, break the chain of radical reactions by converting free radicals into non-radical products. Since it is in close contact with the cornea, anterior chamber, trabecular meshwork, and lens, the RP capacity in the AH has an impact on their health [16].

Changes in GPx activity could significantly impact the steady state concentration of H_2O_2. H_2O_2, which is typically present in the AH and harmful to cells. Although H_2O_2 is a vital part of many signal-transduction pathways, the antioxidant enzymes catalase and GPx eliminate it when its levels rise over healthy limits. Due of its capacity to decrease both inorganic and organic peroxides, GPx may have a higher level of oxidant homeostasis than catalase.

This could result in glutathione depletion because GPx needs glutathione as a cofactor to remove H_2O_2. Ascorbic acid metabolism depends on glutathione, and its depletion results in ascorbyl radicals that cannot be converted back into ascorbic acid [17,18]. Our findings support this theory since oxidative stress brought on by elevated levels of free radicals decreases GPx activity and RP (Table 1).

With the progression of age-related cataracts, from incipient to mature, the capacity of the lens to stimulate hydroxyl radical production increases (Table 2) and dramatically change antioxidant status of the AH.

Free translation metal ions and iron complexes in hemoglobin, myoglobin, lactoferrin, and transferrin may catalyze a variety of pathogenic processes [19]. The concentration of iron and copper ions is lower in lenses without cataracts, while the increase in their concentration affects the increase in the production of hydroxyl radicals (HO•). *In vitro* systems have been

used extensively to study their capacity for creating hydroxyl radicals through the Fenton reaction ($H_2O_2 + M^{(n-1)+} \rightarrow HO^{\bullet} + OH^- + Mn^{n+}$). This suggests that hydroxyl radical damage plays a role in the development of age-related cataract [20]. Also, oxidation of lipoprotein increase [21], while Cu, Zn-SOD activities decreases due to the accelerated generation of ROS, especially hydroxyl radicals. When enzyme activities are lost or diminished, H_2O_2 and free radicals can cause the lens to irreversibly degrade, including a decrease in Na-K ATPase activity [22].

In this work, we examined the direct generation of hydroxyl radicals in AH at different stages of cataract severity. Our results direct evidence that the largest concentration of hydroxyl radicals detected. In all patients with age-related cataract (C, N and M group) Increase in the concentration of hydroxyl radicals in AH causes the process of oxidation (Figure 2).

During aging the content of ascorbic acid decline and their transport into the ocular humor is difficult. Ascorbyl radicals, which have minimal reactivity, are created when ascorbic acid reacts with oxygen radicals. In a nutshell, an increase in oxidative stress is correlated with an increase in ascorbyl radical concentration. In brief, an increase in ascorbyl radical concentration correlates with an increase in oxidative stress.

Several factors need to be considered in order to understand the biochemical mechanisms that may underlie this observation. The reaction of ascorbate monoanion ($AscH^-$) with superoxide anion radical leads to the formation of ascorbyl radical ($AscH^{\bullet -}$). But the reactivity of the superoxide anion is insufficient to explain the damage observed in biological systems. However, many of the harmful effects of superoxide anion are indirect and result from its chemical transformation into a hydroxyl radical. Because of that we investigated the presence of hydroxyl radical in AH of patients with age-related cataract incipient (groups C and N) and mature (Table 2, Figure 4). The hydroxyl radical ($^{\bullet}OH$) is a potent oxidizing agent with very high-rate constants (10^9–10^{10} M^{-1} s^{-1}) for H-abstraction on this reaction $^{\bullet}OH + AscH^- \rightarrow$

$Asc^{-\bullet}+H_2O$. Additionally, as a cataract develops, ascorbic acid loss pathways through ROS and oxidized metals (Cu^{2+} or Fe^{3+}) reactions are potentially conceivable [23]. All proposed mechanisms produce ascrbyl radicals which form dehydroascorbic acid (DHA) as an end product of oxidation. Based on this, a hypothetical model of free radicals in the AH of patients with age-related cataract based on the results of the current study was constructed (Figure 6).

Lipid peroxidation has been proposed as a causative factor of cataract, which will be the subject of our next investigations and also further study is needed to establish some other free radicals which is included into the pathogenesis of age-related cataract. Although this study is limited by small sample size, the results show that the majority of AH oxidative stress markers can be connected to the maturity stage of age-related cataract.

CONCLUSION

The AH protects the inner parts of the eye against the damaging effect of reactive oxygen species generated by them. This is possible due to the effective antioxidant protective mechanisms. According to our results, the RP and GPx activity concentrations were significantly reduced, while the intensities of ESR signals of hydroxyl and ascorbyl radicals increase during the aging process and depend on the maturity of the cataract. AH oxidative stress markers and antioxidants are believed to mirror the intrinsic oxidant/antioxidant balance of the surrounding eye tissues. The presented results suggest that the maturity of cataract should be taken into account in biochemical studies of ocular oxidative stress.

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Table 1. The levels of reducing power and glutathione peroxidase activity in the aqueous humor of patients with age-related cataracts of different maturity

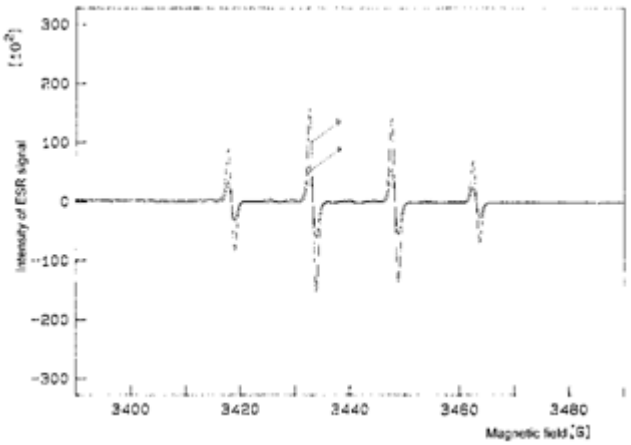
Type of age-related cataract	**Reducing power (mmolTE/ml)**		**Glutathione peroxidase (U/ml)**	
	Mean age (years)			
	67.41 ± 2.89	78.23 ± 3.42	67.41 ± 2.89	78.23 ± 3.42
Cortical cataract (C group)	1.81 ± 0.07[a]	1.02 ± 0.04[a]	4.01 ± 0.14[a]	2.81 ± 0.13[a]
Nuclear cataract (N group)	0.76 ± 0.04[b]	0.66 ± 0.03[b]	2.15 ± 0.07[b]	1.72 ± 0.06[b]
Mature cataract (M group)	0.32 ± 0.08[c]	0.15 ± 0.01[c]	1.05 ± 0.04[c]	0.98 ± 0.03[c]

The values are represented as mean ± standard deviation; values sharing the same letters in the same column are not significantly different from each other at the level $p < 0.05$

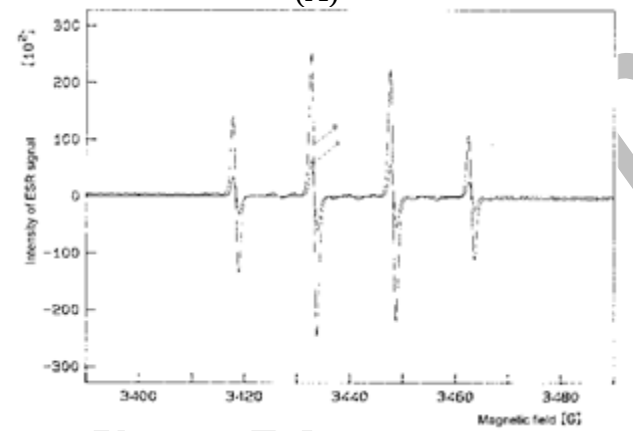
Table 2. Increase in the intensity of the electron spin resonance signal of hydroxyl ($^{\bullet}OH$) and ascorbyl ($ASC^{\bullet-}$) radicals in the aqueous humor of patients with age-related cataracts of different maturity

Type of age-related cataract	**Hydroxyl radical – RI $^{\bullet}_{OH}$(%)**		**Ascorbyl radical - RI$_{ASC^{\bullet-}}$(%)**	
	Mean age (years)			
	67.41 ± 2.89	78.23 ± 3.42	67.41 ± 2.89	78.23 ± 3.42
Cortical cataract (C group)	29.45±1.01[a]	36.22±1.82[a]	26.12±0.89[a]	36.2±1.82[a]
Nuclear cataract (N group)	38.12±1.29[b]	48.72±1.71[b]	41.15±1.39[b]	58.75±2.06[b]
Mature cataract (M group)	74.14±2.52[c]	85.50±1.94[c]	83.56±2.84[c]	90.65±2.96[c]

The values are represented as mean ± standard deviation; values sharing the same letters in the same column are not significantly different from each other at the level $p < 0.05$



(A)



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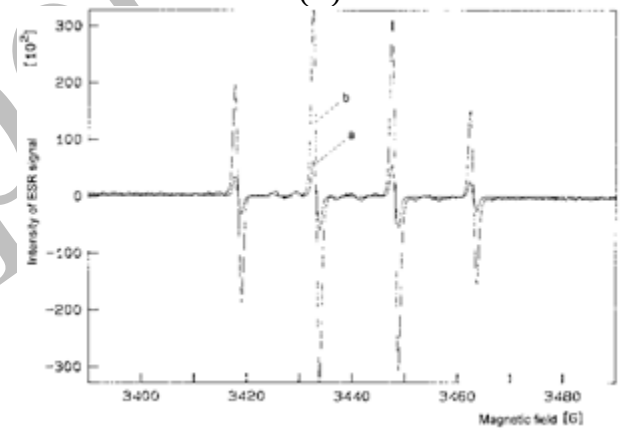

(C)

Figure 1. Electron spin resonance (ESR) spectra of DMPO-OH spin-adduct: A) a) control sample b) aqueous humor of patient P15 with age-related cataract cortical B) a) control sample b) aqueous humor of patient with age-related cataract nuclear P31 C) a) control sample b) aqueous humor of patient with age-related cataract mature P50

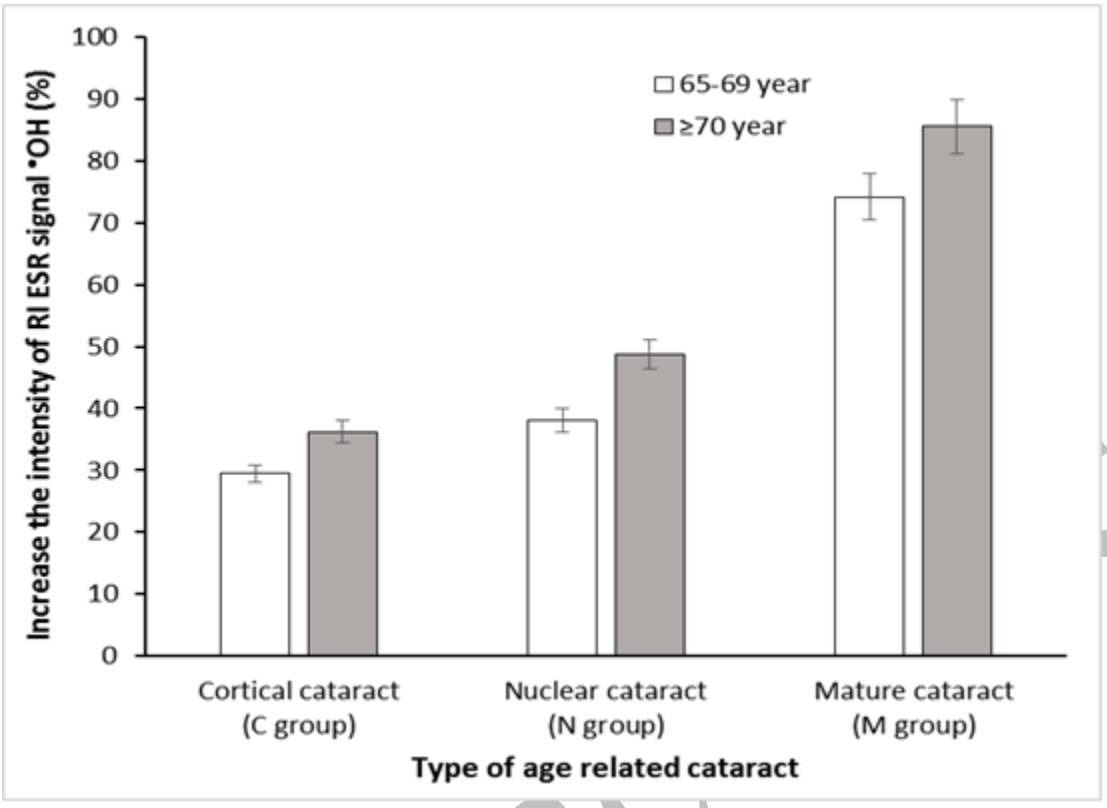

Figure 2. Mean values of the percentage increase in the intensity of the electron spin resonance signal of DMPO-OH spin-adduct during the Fenton reaction in the presence of aqueous humor of patients with age-related cataracts of different maturity compared to the control sample

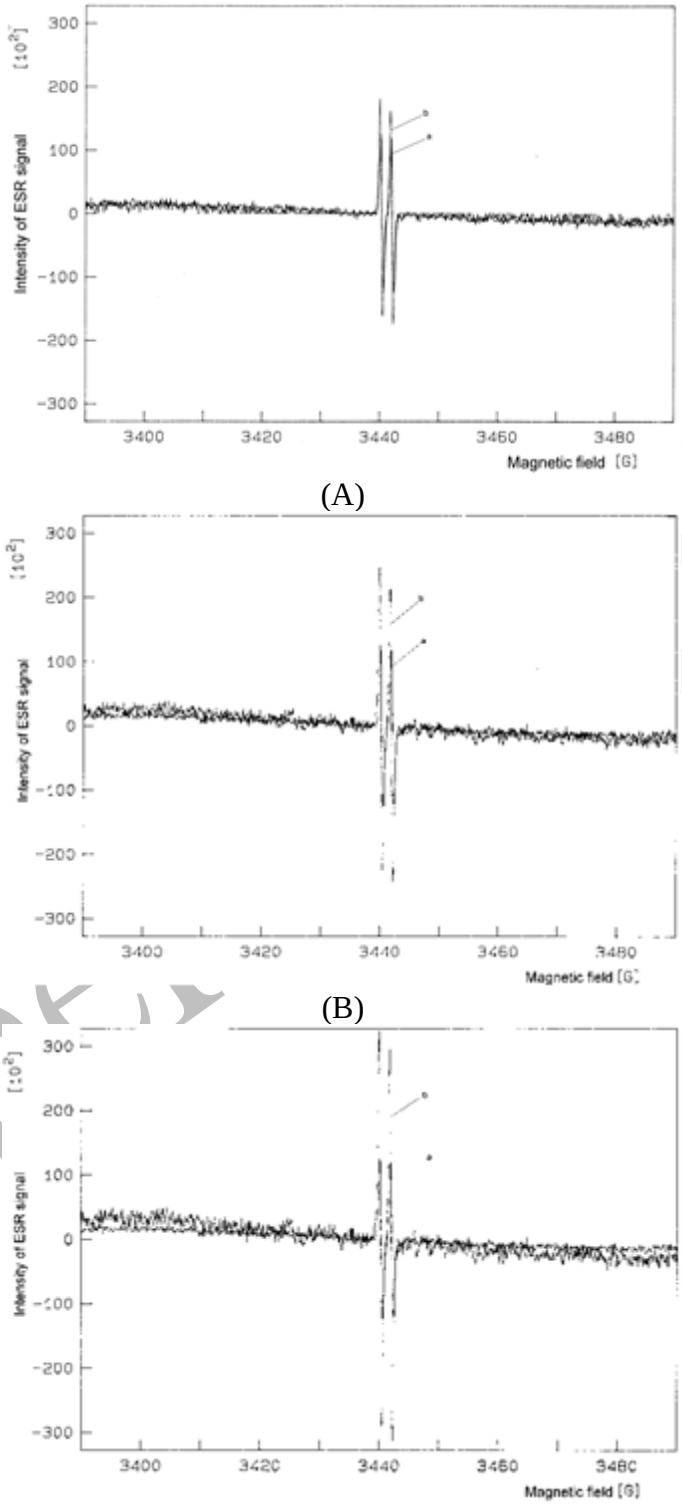

Figure 3. Electron spin resonance (ESR) spectra of ascorbyl radical: A) a) control sample b) aqueous humor of patient P15 with age-related cataract cortical B) a) control sample b) aqueous humor of patient P31 with age-related cataract nuclear C) a) control sample b) aqueous humor of patient P50 with age-related cataract mature

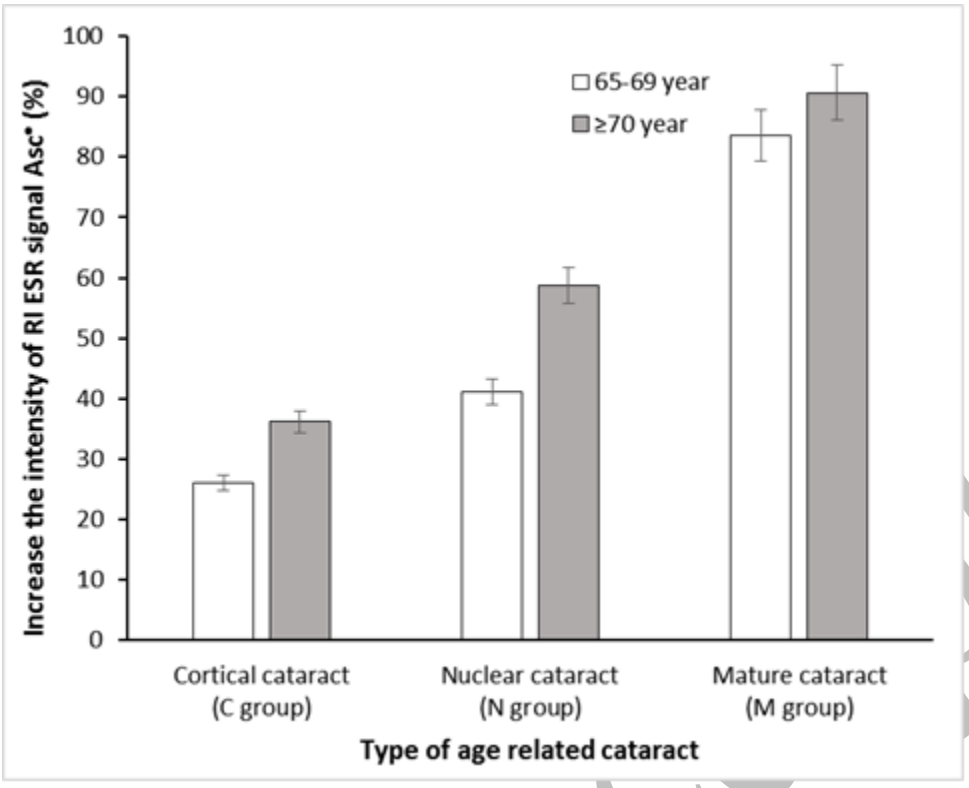

Figure 4. Mean values of the percentage increase in the intensity of the electron spin resonance (ESR) signal of ascorbyl radical obtained in the presence of aqueous humor of patients with age-related cataracts of different maturity compared to the control sample

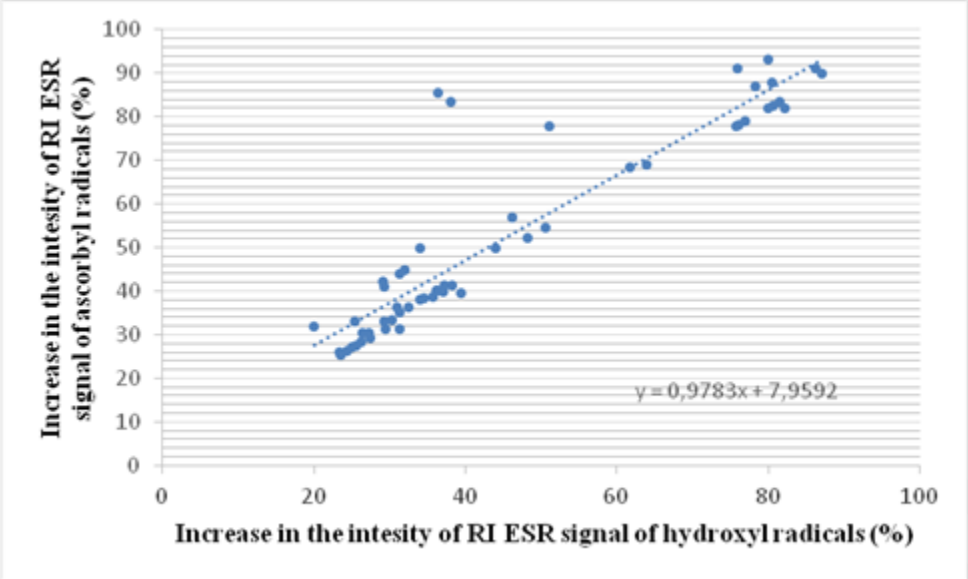

Figure 5. Correlation between the concentrations of ascorbyl radical and the concentrations of hydroxyl radical in aqueous humor of patient with age-related cataracts of different maturity

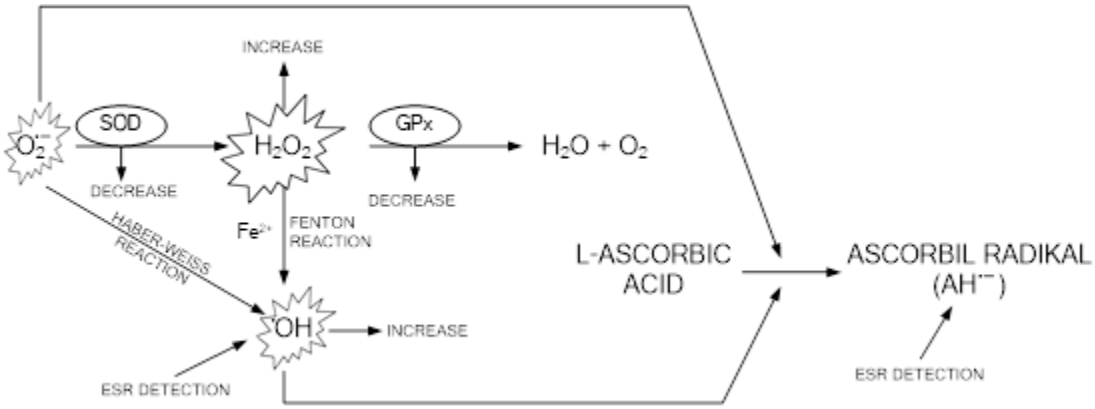

Figure 6. Scheme of free radical/antioxidant imbalance in the aqueous humor of patients with age-related cataract

