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THE IMPACT OF SENILE CATARACT MATURITY ON BLOOD OXIDATIVE STRESS MARKERS AND GLUTATHIONE-DEPENDENT ANTIOXIDANTS: RELATIONS WITH LENS VARIABLES

UTICAJ MATURITETA SENILNE KATARAKTE NA SISTEMSKE MARKERE OKSIDACIONOG STRESA I GLUTATION-ZAVISNE ANTIOKSIDANSE: ODNOS SA SOČIVNIM VARIJABLAMA

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Summary: Oxidative stress is implicated in senile cataract (SC) genesis, although the impact of SC maturity on blood oxidative stress markers is unclear. Total hydroperoxides, malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) were measured in the blood and lens samples of patients having either immature (n=31) or mature SC (n=50), and in 22 blood samples from non-cataract controls. Compared to controls, SC patients had higher plasma MDA and serum GST, and decreased plasma GR and GSH levels. Plasma GPx as well as hydroperoxides differed from control values only in patients with mature SC. The multivariate logistic regression analysis showed that the fall of plasma GR activity (OR 5.14; CI 1.82–14.51; p=0.0020), as well as serum GST activity (OR 3.84; CI 1.36–10.83; p=0.0108) were independently associated with the maturity of SC. Lens hydroperoxides, MDA and GST, showed no correlation with correspondent blood values, in contrast to GPx (r=0.715; p<0.001) and GR (r=0.703; p<0.001). This study showed that the severity of SC is associated with increased systemic oxidative stress, which could be due to the fall of GSH-dependent antioxidant enzymes activities.

Keywords: glutathione-dependent antioxidants, oxidative stress, senile cataract maturity

Kratak sadržaj: Iako oksidacioni stres ima značajnu ulogu u razvoju senilne katarakte (SK), nedostaju dokazi o uticaju stepena maturiteta na sistemske markere oksidacionog stresa. Određivani su ukupni hidroperoksidi i malondialdehid (MDA), kao i glutation (GSH), glutation peroksidaza (GPx), glutation reduktaza (GR) i glutation S-transferaza u krvi i sočivima pacijenata sa nezrelom (n=31) i zrelom SK (n=50), kao i krvi 22 zdrave osobe. U odnosu na kontrolne vrednosti, MDA u plazmi i GST u serumu su bili veći, a vrednosti GR, GSH u plazmi, askorbata i ukupnih tiol jedinjenja bile su niže kod pacijenata sa SK. Plazma GPx i hidroperoksidi razlikovali su se od kontrolnih vrednosti samo kod grupe sa zrelom SK. Multipla logistička regresija pokazala je da su pad aktivnosti GR u plazmi (OR 5,14; CI 1,82–14,51; p=0,0020), kao i pad aktivnosti serumske GST (OR 3,84; CI 1,36–10,83; p=0,0108) nezavisni prediktori maturiteta SK. Interkompartmentalna korelacija ukupnih hidroperoksida, MDA i GST nije bila statistički značajna, za razliku od GPx (r=0,715; p<0,001) odnosno GR (r=0,703; p<0,001). Prezentovani rezultati pokazuju da je veći stepen maturiteta SK povezan sa sistemskim oksidacionim stresom, u čijoj bi intenzifikaciji ulogu mogao imati pad aktivnosti GSH-zavisnih oksidanasa.

Ključne reči: glutation-zavisni antioksidansi, maturitet senilne katarakte, oksidacioni stres

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List of abbreviations: B CDNB – 1-chloro-2, 4-dinitrobenzene; EDTA – ethylenediamino-tetraacetic acid; GSH – glutathione; GR – glutathione reductase; GPx – glutathione peroxidase; GST – glutathione S-transferase; MDA – malondialdehyde; NADPH – nicotinamide adenine dinucleotide phosphate, reduced form; TRIS – 2-amino-2-hydroxymethyl-propane-1,3-diol.

Introduction

An increasing body of evidence suggests that ocular oxidative stress, defined as an imbalance between oxidants and antioxidants, is the key pathophysiological mechanism of senile cataract (SC) genesis (1–5). Hydrogen peroxide is the major ocular oxidant, and can be formed in excess during the photooxidation of endogenous ascorbate and structural lens proteins, the crystallins (1). Exposure of the lens to hydrogen peroxide is well known to cause lipid peroxidation (LPO) of polyunsaturated fatty acids, loss of antioxidants, and oxidation, cross-linking and insolubilization of crystallins. The resulting light scattering and lens opacification generally resemble the changes seen in human SC (2). Some other oxidants like LPO adducts, including organic hydroperoxides and malondialdehyde (MDA), also have significant cataractogenic potential (1), and although they can be present in systemic circulation at micromolar levels and act as toxic messengers, the reports regarding the blood oxidative stress markers in patients with SC are largely inconsistent (5–11).

Tripeptide glutathione (GSH) and the enzymes of its redox-cycle, glutathione peroxidase (GPx, EC 1.11.1.9), glutathione S-transferase (GST, EC 2.5.1.18) and glutathione reductase (GR, EC 1.8.1.7) have important roles in the complexly interrelated network of cellular and extracellular antioxidants. The avascular lens contains an unusually high concentration of GSH which spontaneously, or as a cofactor of GPx and GST, reduces a variety of intracellular oxidizing species. Organic peroxides present in the circulation are, however, converted to correspondent alcohols, mainly by the plasma GPx-3 isoenzyme, a seleno-glycoprotein secreted to the blood by renal proximal tubules, but also expressed within the lens (12, 13). Further solubilization of various epoxides, peroxides and lipid-derived aldehydes via GST-catalyzed conjugation with GSH enhances their removal from the blood. Of the several GST classes, only μ and π are significantly expressed in the lens (14). Glutathione disulfide formed during antioxidant and reductive reactions is reduced back to GSH at the expense of cytosolic NADPH. The reaction is catalyzed by GR, an intracellular homodimeric flavoprotein that is abundantly present in many cells and especially in the lens superficial layers (15). GR is also involved in the maintenance of vitamin C in its reduced state as ascorbic acid (ascorbate), which is the principal non-enzymatic antioxidant of the blood plasma. Incompletely oxidized vitamin C is uptaken in cells and reduced back to ascorbate by GSH, which acts either directly or as a cofactor of glutaredoxin and erythrocyte membrane cytochrome b561 (16). This prevents the formation of irreversible, completely oxidized diketogulonic acid and its loss from the body.

If untreated, SC eventually leads to blindness. Surgical extraction of the opaque lens with intraocular implantation of an artificial lens is currently the only way of treatment, despite of experimental evidence that antioxidant vitamins such as ascorbate, carotenoides and riboflavin may have an anti-cataractogenic effect. Previous studies, including those from our laboratory, have shown that lenticular oxidative stress largely corresponds to the severity of SC (1–4). This relationship with systemic oxidative stress is not well established, although it can be implicated in the severity of several senescence-related diseases and risk factors for SC genesis. Lens GSH-dependent antioxidant enzymes are possible targets for the non-surgical treatment of SC, and their systemic activities could be used as surrogates for lens variables. Little is known, however, about whether blood GSH-dependent antioxidants and oxidative stress markers actually mirror their lenticular counterparts, and how they change during SC progression. To address these issues, we assessed MDA and total hydroperoxides as LPO markers, total protein SH-groups as a marker of protein oxidative damage, and GSH-dependent antioxidants in paired lens and blood specimens from patients having either immature or mature SC.

Subjects and Methods

Study groups

This study included consecutive patients who underwent planned cataract surgery with intraocular lens implantation. Clinical type of SC and the maturation stage of opacity were determined by routine ophthalmologic examination and confirmed during surgery. Patients with hypermature SC or having ocular diseases other than SC, as well as with known chronic hepatic, renal or metabolic diseases, were not included. However, patients having hypertension (HTA), defined as systolic blood pressure ≥140 mm Hg, or on anti-hypertensive medication, were included. There were 81 eligible patients (81 eyes), 33 females and 48 males (mean age 66.2±5.3 years). The control group consisted of 22 blood samples from non-cataract subjects routinely checked-up for presbyopia, who met the same exclusion criteria (10 females and 12 males; mean age 65.4±4.0 years). Informed consent was provided after careful explanation of the aims of the study. This research was conducted following the tenets of the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty Pristina (Kosovska Mitrovica).

Sample collection and preparation

The lens (nucleus plus cortex), obtained during extracapsular cataract extraction, was rinsed with a cold physiological saline solution and transported to the laboratory packed on ice. Lens was briefly homogenized in a ten-fold volume (w/v) of cold buffer (0.2 mol/L potassium phosphate; 137 mmol/L potassium

chloride; 60 mmol/L sodium dodecyl sulfate; pH 7.2), and spun (12 000 × g; 40 °C; 20 minutes). A clear supernatant was used for biochemical assays.

Blood was taken preoperatively after overnight fasting into a vacutainer tube with or without heparin. Plasma and serum were available after routine biochemical analyses were completed.

Biochemical measurements were carried out in a UV/VIS spectrophotometer (Safas 2, Monaco) fitted with constant temperature cell housing. Except for total SH-groups, the other lens biochemical variables were expressed in correspondent units per gram tissue wet weight.

Measurement of MDA

MDA in the lens and plasma samples was determined as described (17). Absorbance readings of the pinkish trimethin complex formed in reaction with 2-thiobarbituric acid (2-thiobarbituric acid, 70 mmol/L; NaOH, 50 mmol/L) were taken at $\lambda = 532$ nm against reagent blank. Concentration of MDA was calculated using the molar extinction coefficient of $\lambda = 1.56 \times 10^5$ L × mol^{-1} × cm^{-1}.

Determination of total hydroperoxides

The concentration of lens and plasma hydroperoxides, as an early marker of LPO, was measured by the ferrous-oxidation xylenol orange method (FOX2), after reduction of the preexisting peroxides with triphenylphosphine (TPP) (18). In brief, 90 μL of sample was mixed with 10 μL HPLC-grade methanol, or 10 μL TPP solution (TPP 20 mmol/L; in methanol), and left at room temperature in a dark place for 30 minutes before adding 900 μL of FOX solution (250 μmol/L ammonium ferrous sulfate, 100 μmol/L xylenol orange, 25 mmol/L sulfuric acid, and 4 mmol/L butylated hydroxytoluene in 90% (v/v) methanol, in a final volume of 100 mL). After 30 minutes at room temperature, samples were vortex mixed and spun for 10 minutes at 10 000 × g. The resulting ferric-xylenol orange complex was measured at 560 nm against blank probe prepared with FOX-blank reagent in which the xylenol orange was omitted. Concentration of total hydroperoxides was calculated as the difference between untreated and TPP-treated samples, and calibrated with hydrogen peroxide standard curve freshly prepared in the concentration range of 0–25 μmol/L.

Measurement of total sulfhydryl groups

Lens and plasma total sulfhydryl groups (SH-groups), as an indicator of oxidative protein damage, were measured with Ellmans's reagent (19). 20 μL of sample was mixed with 800 μL sodium phosphate (Na_2HPO_4; 0.3 mol/L) and 200 μL of Ellman's reagent (40 mg of 5, 5'-dithiobis-(2-nitrobenzoic acid) in 100 mL of 1% (w/v) sodium citrate). After 10 minutes the absorbance was measured at $\lambda = 412$ nm against reagent blank. The amount of total SH-groups was calculated using the molar absorbance of 1.36×10^4 L × mol^{-1} × cm^{-1}.

Measurement of GSH content

Lens and plasma GSH concentration was measured with Ellmans's reagent after sample deproteinization (19). 0.2 mL of sample was added to tubes containing 0.2 mL of 1 mol/L perchloric acid supplemented with 2 mmol/L di-sodium EDTA, vortex-mixed and spun for 5 minutes at 10 000 × g (40 °C) to remove protein precipitate. A clear supernatant (200 μL) was mixed with 0.3 M sodium phosphate (800 μL) and Ellman's reagent (200 μL), and after 10 minutes the absorbance was measured at $\lambda = 412$ nm against reagent blank. Concentration of GSH was calculated using the molar absorbance of 1.36×10^4 L × mol^{-1} × cm^{-1}.

Glutathione peroxidase activity assay

GPx activity in the lens and plasma samples was measured by the colorimetric method using cumene hydroperoxide as a substrate (20). In brief, 25 μL of sample was mixed with 375 μL TRIS-HCl buffer (50 mmol; pH 7.6; supplemented with 5 mmol/L sodium azide to block catalase). The reaction was started by addition of 50 μL freshly prepared GSH (6 mg in 10 mL of 50 mmol/L TRIS-HCl buffer, pH 8.9) and 50 μL cumene hydroperoxide (3 mmol/L, in concentrated methanol). After exactly 5 minutes at 37 °C the reaction was stopped by addition of 0.5 mL 20% trichloroacetic acid (w/v), and spun to remove protein precipitate. Concentration of GSH retained in a clear supernatant was determined with Ellmans's reagent, as described above. The blank probe was introduced to correct the oxidation of GSH with cumene hydroperoxide in the absence of GPx. One unit of GPx activity was defined as the amount of enzyme that catalyses oxidation of 1 μmol/L of GSH per minute, at 37 °C.

Glutathione S-transferase activity assay

GST activity in the lens and serum samples was measured with 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate, as reported by Habdous and co-workers (21). The reaction mixture contained 5 mmol/L GSH, 2.5 mmol/L CDNB and 3 mg/mL of bovine serum albumin in 0.1 mol/L potassium phosphate buffer (pH 5.5). Formation of GSH-CDNB conjugate was continuously monitored for 3 minutes at $\varepsilon = 340$ nm (30 °C), following addition of 80 μL of sample to start the reaction. Enzyme activity was calculated using the molar

absorbance of $\varepsilon = 9.6 \times 10^3 \times L \times mol^{-1} \times cm^{-1}$. One unit of GST activity was defined as the amount of enzyme catalyzing the formation of 1 μmol/L of GSH-CDNB conjugate per minute, at 30 °C.

Glutathione reductase activity assay

GR activity in the lens and plasma samples was measured by the rate of NADPH consumption, as described by Racker (22). Briefly, 20 μL of sample was introduced to the reaction mixture (pH 7.0) consisting of 100 mmol/L potassium buffer, 1 mmol/L EDTA, 1 mmol/L glutathione disulfide and 0.2 mmol/L NADPH (dissolved in 0.1% $NaHCO_3$). The change in optical density was monitored at $\lambda = 340$ nm at 37 °C for 3 minutes, and corrected for spontaneous oxidation of NADPH. One unit of GR activity was defined as 1 μmol of NADPH oxidized per minute, at 37 °C.

Other biochemical measurements

Total plasma vitamin C and ascorbic acid concentrations were measured after sample deproteinization (60 g/L meta-phosphoric acid; 2 mmol/L disodium EDTA) using a freshly prepared 2,4-dinitrophenylhydrazine-thiourea-copper (II) sulfate working reagent (23); for the ascorbic acid assay copper sulfate was omitted from the working reagent. Ascorbic acid was calculated as the difference of values with and without added copper sulfate, and calibrated against an ascorbic acid standard curve freshly prepared in the concentration range of 15–240 μmol/L. Total protein concentration in the lens water-soluble fraction was determined by the method of Lowry (24).

Statistical analysis

Data distribution and homogeneity of variance were tested by Kolmogorov-Smirnov test. The data without Gaussian distribution were logarithmically transformed before analysis, and back-transformed for data presentation. Differences between numerical data were tested by Student's t-test for independent samples, or by ANOVA and Bonferroni's *post hoc* test. Categorical data were tested by chi-square test. Correlation analysis was accomplished by the calculation of Pearson's correlation coefficient. Multivariate logistic regression analysis with stepwise elimination was performed to estimate odds ratios (OR) and 95% confidence intervals (CI) for associations between cataract severity and lens or blood biochemical variables as independent predictors. Principal components and classification analysis (STATISTICA software package; Statsoft, Tulsa, USA) was performed in order to assess the interrelations between multiple individual variables in cataract. This method is designed to reveal any inherent trend or pattern in the data set. Statistical significance was set at $p<0.05$.

Results

Basic clinical and blood biochemical characteristics of 81 cataract patients and 22 control subjects are summarized in *Table I*. The immature cataract group (n=31) consisted of cortical (n=7), posterior subcapsular (n=6), nuclear (n=7), and mixed (nuclear and cortical; n=11) opacities; the second group (n=50) was with white mature cataract. There were no statistically significant differences between the SC

Table I Clinical and blood biochemical characteristics of senile cataract patients and control subjects.

	Immature cataract (n = 31)	Mature cataract (n = 50)	Controls (n = 22)
Age (years)	65.9 ± 5.9	66.8 ± 4.9	65.4 ± 4.0
Gender (female/male; n)	13/18	20/ 30	10/12
HTA (yes/no; n)	9/22	16/ 34	9/13
Cigarette smoker (yes/no; n)	6/25	7/ 43	6/16
MDA (μmol/L)	4.55 ± 0.97[a]	5.11 ± 1.23[a, b]	3.72 ± 0.75
Hydroperoxides (μmol/L)	6.65 ± 1.39	7.21 ± 1.50[a]	6.17 ± 1.67
Total SH-groups (μmol/L)	397 ± 60[a]	372 ± 57[a]	474 ± 73
Total vitamin C (μmol/L)	64.2 ± 4.6	64.7 ± 5.2	65.1 ± 4.4
Ascorbic acid (μmol/L)	27.1 ± 4.1[a]	26.2 ± 3.9[a]	35.7 ± 5.4
GPx (U/L)	40.5 ± 5.7	34.1 ± 7.8[a, b]	43.6 ± 6.4
GST (U/L)	46.7 ± 8.6[a]	39.9 ± 5.4[a, b]	31.3 ± 6.3
GR (U/L)	22.2 ± 3.2[a]	17.4 ± 3.1[a, b]	28.7 ± 5.1
GSH (μmol/L)	5.94 ± 1.43[a]	6.19 ± 1.34[a]	7.72 ± 1.77

Data are presented as means ± SD or frequencies (n). Hypertension (HTA) was defined as systolic blood pressure ≥ 140 mmHg, or on anti-hypertensive medication. [a]$p < 0.05$, compared to controls, and [b]$p < 0.05$, for comparisons between cataract groups, after ANOVA and Bonferroni *post hoc* test.

groups and control subjects regarding age and gender distribution, the presence of HTA and smoking habits. Plasma GPx activity was slightly lower in the SC patients previously diagnosed with HTA (n=25) than in those without HTA (n=56), and was 35.1±8.1 U/L vs. 38.1±6.4 U/L, in HTA and non-HTA, respectively (p=0.077). No such difference existed among the control subjects (p=0.963).

Blood biochemical variables adjusted for age, gender, HTA, and smoking were further analyzed as presented in *Table I*. In comparison to controls, plasma MDA was higher in immature (p=0.002), and in mature SC (p<0.001), with a significant difference between the cataract groups (p=0.034). Plasma hydroperoxides were higher than in controls only in the group with advanced SC (p=0.011), while total plasma SH-groups were decreased in both SC groups, as were plasma ascorbate and GSH levels (p<0.001, respectively). Total vitamin C concentrations did not differ from controls in either the immature (p=0.478) or mature SC group (p=0.754). In comparison to controls, plasma GPx activity was decreased in immature (p=0.068), and in advanced SC (p<0.001), with a significant difference between the cataract groups (p<0.001). Plasma GR activity also decreased with the severity of disease, and the differences between controls and each SC group, as well as between patient groups were significant (p<0.001, respectively). In comparison to the control values, serum GST activity was significantly higher in both cataract groups, and higher in immature than in advanced cataract (p<0.001, respectively).

Lens biochemical variables adjusted for confounding factors are presented in *Table II*. In comparison to the immature, lenses with mature SC had a 20% lower content of water-soluble proteins (p=0.001),

Table II Lens biochemical variables in senile cataract.

	Immature cataract (n = 31)	Mature cataract (n = 50)
Wet weight (mg/lens)	140 ± 41	138 ± 34
Water-soluble proteins (mg/g lens)	82.5 ± 17.7	69.3 ± 14.8[a]
MDA (nmol/g lens)	36.8 ± 9.6	61.5 ± 8.4[a]
Hydroperoxides (nmol/g lens)	60.2 ± 11.1	77.6 ± 8.3[a]
GSH (μmol/g lens)	1.59 (1.34–1.97)	0.42 (0.37–0.47)[a]
GPx (U/g lens)	0.91 (0.62–1.36)	0.47 (0.42–0.54)[a]
GST (U/g lens)	0.58 ± 0.14	0.48 ± 0.13[a]
GR (U/g lens)	0.70 ± 0.22	0.53 ± 0.18[a]
Total SH-groups (μmol/g protein)	100.8 ± 22.7	56.7 ± 19.3[a]

Biochemical variables are presented as means ± SD, or geometric means (¶) and 95% CI for the mean (in parenthesis); [a]p<0.05, compared to immature cataract group after Student's t-test for independent samples.

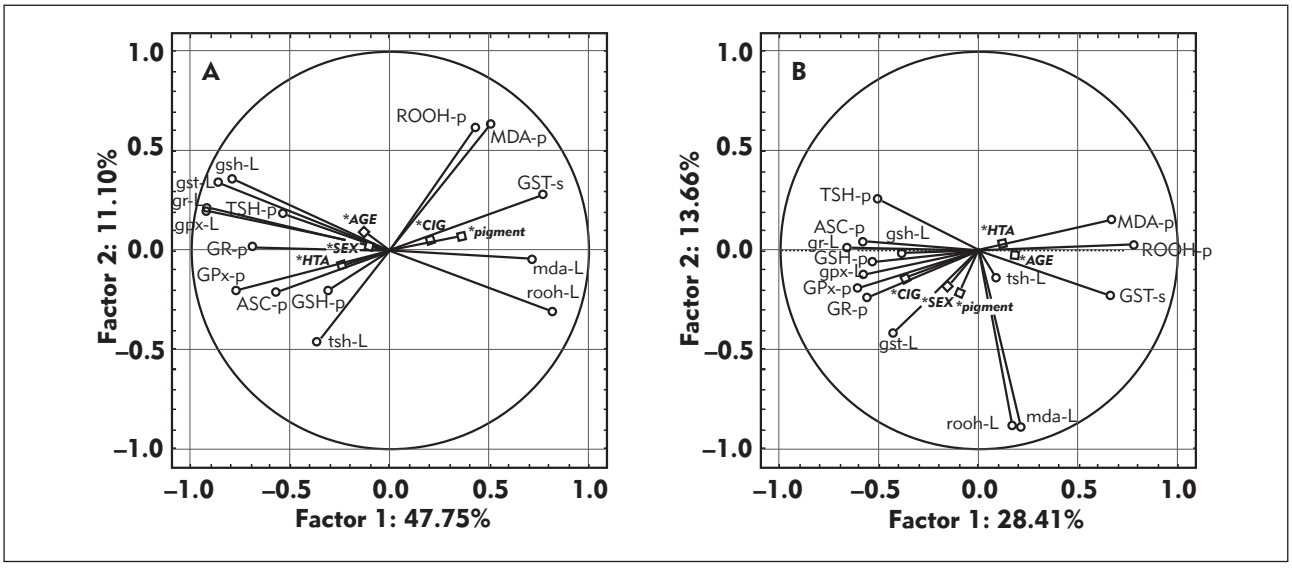

Figure 1 Relationships between lens and blood oxidative stress markers and antioxidants in immature (*Figure 1A*) and mature (*Figure 1B*) senile cataract estimated by principal component and classification analysis. Lens (L), plasma (p) and serum (s) variables were evaluated by principal component and classification analysis taking age, sex, hypertension (HTA), cigarette smoking (CIG) and lens pigmentation (pigment) as supplementary variables. Lowercase letters – lens variables; uppercase letters – plasma/serum variables; ROOH (rooh) – total hydroperoxides; MDA (mda) – malondialdehyde; TSH (tsh) – total thiol groups; GSH (gsh) – glutathione; GPx (gpx) – glutathione peroxidase; GR (gr) – glutathione reductase; GST (gst) – glutathione S-transferase; ASC – plasma ascorbate.

and about 2 times lower total SH-groups, GR, GPx and GST ($p<0.001$, respectively). Moreover, the GSH content was 3 to 10 times lower in lenses with advanced disease ($p<0.001$). On the other hand, lenses with mature SC had higher hydroperoxide and MDA levels than those with immature cataract ($p<0.001$, respectively).

Associations between lens and blood biochemical variables at the different maturation stages of SC were evaluated by principal components and classification analysis, taking age, gender, HTA, cigarette smoking and lens pigmentation as supplementary variables (*Figure 1*). Biochemical variables formed two distinctive clusters in immature cataract (*Figure 1A*): the first one consisted of lens and blood LPO markers plus serum GST; the second cluster represented lens and blood GSH-dependent antioxidants plus plasma ascorbate. In advanced SC the latter retained its position and structure (*Figure 1B*), while the first was split into lens LPO markers, and blood LPO markers plus serum GST.

Relationships between blood antioxidant enzymes and the lens and blood markers of oxidative injury were further evaluated. In overall samples, plasma GPx activity was inversely correlated with lens and plasma hydroperoxides ($r=-0.35$; and $r=-0.46$; $p<0.01$, respectively) and plasma MDA ($r=-0.497$; $p<0.001$), and positively correlated with lens and plasma total SH-groups ($r=0.46$; and $r=0.36$; $p<0.001$, respectively). Plasma GR activity was inversely correlated with lens hydroperoxides and MDA ($r=-0.51$; and $r=-0.39$; $p<0.001$, respectively), and also with plasma hydroperoxides and MDA ($r=-0.39$, and $r=-0.35$; $p=0.01$, respectively). Plasma GR activity was positively correlated with lens and plasma total SH-groups ($r=0.53$; and $r=0.40$; $p<0.001$, respectively). In the immature SC group, serum GST activity was positively correlated with lens hydroperoxides ($r=0.49$; $p=0.005$), plasma hydroperoxides ($r=0.45$; $p=0.010$) and plasma MDA ($r=0.57$; $p<0.001$), but inversely correlated with lens total SH-groups ($r=-0.38$; $p=0.036$). In the group with mature SC, serum GST also correlated with plasma hydroperoxides ($r=0.36$; $p=0.010$) and plasma MDA ($r=0.32$; $p=0.015$). Intercompartmental correlations of LPO markers and GSH-dependent enzymes are shown in *Table III*.

The stepwise multivariate logistic regression analysis was performed in order to identify the associations of biochemical variables with cataract maturity. Lens and blood biochemical variables were considered as separate data subsets. Of all the biochemical variables tested, only lens GSH, and plasma GR and serum GST were retained in the respective models as independent predictors of cataract maturity (*Table IV*).

Discussion

It is generally believed that chronic exposure of the lens to environmental and intrinsic oxidants, in conjunction with age-related, genetic and dietary factors, may over time trigger the development of SC. Given that once formed lens opacity inevitably progresses, it was not surprising that the concentrations of lens oxidative stress markers, expressed as total hydroperoxides and MDA, as well as decreased soluble proteins, total SH-groups and GSH-dependent antioxidants, were well correlated in our study with the clinical grading of SC, and largely in accordance

Table IV Multivariate logistic regression for associations of lens and blood oxidative stress markers with senile cataract severity.

	OR	CI	p-value
Lens GSH (μmol/g lens)	17.93	4.04–79.63	0.0001
Plasma GR (U/L)	5.14	1.82–14.51	0.0020
Serum GST (U/L)	3.84	1.36–10.83	0.0108

Table III Intercompartmental correlations of blood oxidative stress markers and GSH-dependent antioxidant enzymes in senile cataract.

	Immature cataract (n = 31)		Mature cataract (n = 50)		All cataracts (n = 81)	
	r	*p-value*	r	*p-value*	r	*p-value*
Hydroperoxides	0.214	0.249	−0.015	0.918	0.079	0.483
MDA	−0.053	0.778	−0.137	0.343	−0.091	0.413
GPx	0.658	< 0.001	0.712	< 0.001	0.715	< 0.001
GST	−0.313	0.086	0.065	0.652	−0.092	0.414
GR	0.677	< 0.001	0.655	< 0.001	0.703	< 0.001

Blood and lens biochemical variables were adjusted for age, gender, HTA and cigarette smoking. Lens GPx was logarithmically transformed before analysis to achieve Gaussian distribution. Intercompartmental correlations are given as Pearson's correlation coefficient (r) and p-value.

with the previous reports (1–5). The underlying reasons of reduced lens GPx, GR and GST activities in advanced disease are highly complex, as can be the result of downregulated or insufficiently expressed genes (14), as well as oxidative modifications of enzymatic molecules, potentiated by extensive loss of the cellular GSH pool (25, 26). As shown in our study, the concentration of lenticular GSH was independently associated with cataract maturity [OR 17.93 (CI 4.04–79.63); p=0.0001], thereby confirming its pivotal antioxidant role in this organ, as emphasized by others (3).

Previous case-control studies were, however, inconclusive about the enrollment of systemic oxidative stress (6, 10), and the possible reasons of the disturbed oxidant/antioxidant balance in SC (5, 7, 8, 9, 11). It has to be noted that the genesis of SC is of multiple origin, and some etiological risk factors, like visible and UV light exposure, have long-lasting but mostly local effects. Thus, the blood oxidative stress markers may not reliably represent changes in the lens organ. In our study, the severity of SC was accompanied by a gradual but modest increase in both plasma LPO markers, none of which was independently associated with the severity of disease. We also found no LPO intercompartmental correlation at either stage of SC. It was previously assumed that this can be a consequence of the different origin of LPO adducts (6), although it may also reflect the compartment-specific metabolic fate of LPO brought about by the differences in antioxidants and export mechanisms. In particular, the lens is an avascular organ with limited efflux of substances to adjacent tissues, which would favor the accumulation of LPO adducts within the lens fibers. As shown, the lenticular concentration of relatively stable MDA was nearly doubled in advanced disease, which was not the case with plasma MDA.

The systemic oxidative stress in SC was previously associated with the decreased activities of superoxide dismutase (7), catalase (8), paraoxonase (9) and GST (11). The majority of blood hydroperoxides are, however, reduced by plasma GPx, and their efficient removal would thus diminish plasma MDA levels as well, as indicated in our study by the significant inverse correlations between plasma GPx, hydroperoxides and MDA. Plasma GPx also expresses a peroxynitrite reductase activity, with implications on redox-signaling and the regulation of vascular tonus (12), which may explain the tendency of SC patients with HTA to have lower plasma GPx than the normotensive ones. In comparison to immature SC, plasma GPx and GR activities were markedly decreased in the mature SC group, with GR activity independently associated with the severity of disease [OR 5.14 (CI 1.82–14.51); p=0.0020]. To the best of our knowledge, the impact of cataract maturity on these enzymes was not previously evaluated, although Leske et al. reported that, in comparison to healthy subjects, SC patients were twice as likely to have lower erythrocyte GR activity (15).

As the key enzyme of the GSH-redox cycle, GR controls many aspects of antioxidant defense including the plasma vitamin C redox state (16). Hence, our findings of decreased plasma ascorbate and GSH, with virtually unchanged total vitamin C levels, argue for inadequate recycling of oxidized vitamin C, rather than the nutritive deficiency. However, a low ascorbate dietary intake was previously associated with systemic oxidative stress and the severity of SC in undernourished Indian patients (5). According to experimental studies, ascorbate- or riboflavin-deficient diet leads to enhanced lens and systemic oxidative stress, and may result in cataractogenesis (27). The impact of dietary antioxidants on the reduction of risk of SC is still unresolved, since most of the controlled clinical trials failed to confirm their beneficial effects (28). Plasma GR and GPx activities were in our study evidently related to the maturation stage of SC, thus their synergistic reduction could also be an epiphenomenon of the disease severity. Physical inactivity, for example, which often accompanies the greater visual disability, has been shown to decrease both kidney and lens GPx and GR in experimental models, as well as erythrocytes GR in healthy elderly people (29–34). Plasma GPx and GR were in our study well correlated with correspondent lens enzymes in either stage of SC, suggesting that they may serve as lens surrogate markers.

Other studies have linked the gene deletion of some members of μ and/or θ GSTs, and thus lower enzyme activity, with increased plasma LPO levels and the risk of SC (11, 13). A recent systematic meta-analysis has, however, largely questioned the relevancy of the GSTs polymorphisms on susceptibility of the Caucasian population to SC development (35). In our study serum GST activity was, quite the opposite, significantly elevated in SC patients, with the highest values found in the immature cataract group. Considering the fact that α and to some extent the other GSTs express selenium-independent peroxidase-like activity (36), it cannot be ruled out that this apparently upregulated systemic response was cytoprotective, contributing to the just moderately increased blood LPO levels in our SC patients. In that way, the finding that serum GST was independently associated with SC maturity [OR 3.84 (CI 1.36–10.83); p=0.0108] supports the assumption of Saadat (13) that the more severe lens opacification could be related to the fall of systemic GST.

The principal component and classification analysis showed interesting relationships. As expected, ocular GSH-dependent antioxidants were inversely correlated with lens hydroperoxides and MDA. Moreover, plasma GPx, GR and GSH were inversely correlated with the lens LPO markers, suggesting their protective roles against lenticular oxidative damage. Surprisingly, however, serum GST was positively corre-

lated with both the lens and plasma LPO markers, and inversely associated with the lens GSH-dependent antioxidants. Although the majority of these findings were also confirmed by correlation analysis, they do not necessarily implicate any causation and can actually reflect an increased induction of systemic GST by the oxidants. Still, it was suggested that some potentially cataractogenic substances could be activated via the mercapturic acid pathway (37) in which the GST has a central role. The lack of correlation between lens and serum GST probably reflects the presence of phenotypes with different affinity for CDNB in these compartments, thus its determination is apparently not a suitable surrogate for lens enzyme activity.

Taken together, these results indicate that the severity of SC is associated with augmented systemic oxidative stress in which the decreased activities of GSH-dependent antioxidants may be implicated. Lower activities of plasma GR as well as serum GST were independently associated with the clinical grading of SC. Given that blood oxidative stress markers are often evaluated as surrogates for lens variables, our results suggest that the clinical grade of SC should be taken into account. Unlike serum GST, plasma GPx and GR were well correlated with the correspondent lens enzymes activities in either stage of SC, and therefore may serve as lens surrogate markers. To conclude, this study supports and extends the idea that the progression of SC is associated with enhanced systemic oxidative stress, and provides evidence that this could be due to the fall of non-ocular GSH-dependent enzymes activities.

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Conflict of interest statement

The authors declare having no conflict of interest related to the publication of this manuscript.

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