

AGE – RELATED MACULAR DEGENERATION: BIOCHEMICAL ASPECTS

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Abstract. Aim: to determine the level of antioxidant enzymes from patient with the age macular degeneration (ARMD), cataract or both ARMD – cataract. **Material and methods:** the patients were diagnosed in the Ophthalmological Clinic from University Clinic No. 1 “Sf. Spiridon” Iași. We studied the antioxidant system superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in order to establish a correlation between these enzymes and the occurrence of ARMD and cataract. The patients with systemic diseases such as: diabetes mellitus, hypertension, hyperlipemia, and also with other ocular diseases were excluded. **Results and discussion:** in the group of patients diagnosed with ARMD superoxide dismutase, catalase and glutathione peroxidase values were statistically significant decreased ($p < 0.01$), compared with the control group. For the patients diagnosed with ARMD and cataract, GPx activity was significantly decreased compared with the control group. **Conclusions:** our study showed that the lower levels of blood natural antioxidant enzymes are related to higher incidence of ARMD or cataract and to the association ARMD and cataract.

Key words: macular degeneration, cataract, antioxidant enzymes

Rezumat. Scop: de a determina nivelul enzimelor antioxidante la pacienții cu degenerescență maculară (DMLV), DMLV și cataractă. **Material și metodă:** Acest studiu se bazează pe particularitățile stress-ului oxidativ în relație cu degenerescența maculară legată de vârstă, cataractă sau de ambele boli, precum și producerea anionului superoxid O_2^- inițiatorul formării speciilor reactive de oxigen și a radicalilor liberi. Pacienții au fost diagnosticați la Clinica Oftalmologică Nr.1, din cadrul spitalului universitar “Sf. Spiridon”, Iași Romania. Am studiat sistemele antioxidante: superoxid dismutaza (SOD), catalaza (CAT) și glutation peroxidaza (GPx) în vederea stabilirii unei corelații între aceste enzime și apariția DMLV și a cataractei. Pacienții cu boli sistemice ca: diabet zaharat, hipertensiune, hiperlipemie precum și cu alte boli oculare au fost excluși. **Rezultate și discuții:** în grupul de pacienți diagnosticați cu DMLV valorile SOD, CAT și GPx au fost semnificativ scăzute ($p < 0,01$) în comparație cu grupul de control. La pacienții cu DMLV și cataractă activitatea GPx a fost mai scăzută comparativ cu grupul de control. **Concluzii:** Studiul nostru demonstrează că nivelul scăzut al enzimelor antioxidante din sânge este în concordanță cu incidența crescută a DMLV, cataractei și a ambelor boli asociate.

Cuvinte cheie: degenerescența maculară, cataractă, enzime antioxidante

INTRODUCTION

Age-related macular degeneration (ARMD) is the leading cause of irreversible severe visual loss in the

industrialized world. Although the exact cause of ARMD is unknown, research suggests age as a risk factor. It is estimated that four to ten million

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people aged between 45 and 75 years suffer from ARMD. The prevalence of severe visual loss increases with age (1). The disease is often bilateral, and patients report a significant history of disease in family members who have lived to later years of their life (2). The macula is the region of the retina that is responsible for the exquisite visual acuity as well as colour vision. Most of the receptors in the macula are cones. There are two main types of ARMD: atrophic (non exudative, dry) and exudative (wet), which both tend to be progressive and bilateral, though they differ in presentation, management and prognosis (3). It is known that free radicals (O_2^- , HO) react easily and could damage both DNA and other cell membranes. The radicals may act directly or indirectly by generating strong oxidizing agents within the cells as a result of metabolic processes or local environmental factors.

Epidemiological studies of diet, environmental and behavioral risk factors suggest that oxidative stress is a contributing factor of ARMD (4).

MATERIAL AND METHODS

The study was performed on 40 subjects (10 men and 30 women) aged of 50 – 80 years old, diagnosed with ARMD, 40 patients (20 men and 20 women) diagnosed with cataract, 35 patients (15 men and 20 women) diagnosed with both ARMD and cataract, comparative to a control group of 30 patients (15 men and 15 women). For comparison purposes healthy volunteers were selected, on the basis of age and BMI below 25 Kg/m^2 . Patients with systemic diseases

such as: diabetes, hypertension, and also other ocular diseases were excluded. Blood samples were obtained in the morning after an overnight fast both from the patients and the control group. At the control group, blood was drawn at the initial moment. The antioxidant enzymatic system (superoxide dismutase, catalase and glutathione peroxidase), was also dosed in the total blood drawn on heparin. SOD was investigated according to the method described by Minami and Yoshikawa (5). The method is based on the inhibition by SOD of pirogallol induced nitroblau tetrazolium reduction. The amount of enzyme that causes 50% inhibition of reduction of nitroblau tetrazolium by pirogallol was defined as 1 unit of SOD activity.

For CAT we have noticed the followings: it is not possible to define international catalase units (U) according to International Unities of Biochemistry (IUB) recommendation. Use of the rate constant of the first order reaction (K) is recommended. The rate constant related to the hemoglobin content (K/Hb) can serve as a measure of the specific activity of erythrocyte catalase (6). Enzymatic activity expressed by K_{15}/g of hemoglobin K is the first order rate constant of the enzymatic decomposition of H_2O_2 (range concentration 0.01-0.05 M) at 240 nm, the rate being proportional to the concentration of peroxide, during about 1.0 min. K_{15} is K at 15 s time interval.

Erythrocyte GPx activity was investigated according to the method described by Fukuzawa (7). The statistical processing of data was made using EPI INFO 6 program and involved means, standard

deviations, “t” test and confidence intervals (CI).

RESULTS AND DISCUSSION

In the group of patients diagnosed with ARMD superoxide dismutase, catalase and glutathione peroxidase values were statistically significant decreased ($p < 0.01$), compared with the

control group (table1). The decrease of the antioxidant system activity, associated with increased macular membrane lipoperoxidation reflected the higher oxidative stress. SOD catalyses the anion superoxide (O_2^-) reduction, one of the most reactive oxygen species, to H_2O_2 , less reactive oxygen species (8).

Table 1. Erythrocyte SOD activity (U/Ht x 100) in cases groups compared to control

	Control group	ARMD group	CATARACT group	ARMD and CATARACT group
Mean value ± SD	7.12 ± 0.16	5.58 ± 0.44	6.76 ± 0.55	5.84 ± 0.77
95% CI	(7.06 - 7.17)	(5.44 - 5.71)	(6.56 – 6.96)	(5.58 – 6.09)
P		≤ 0.01	≤ 0.01	≤ 0.01

Hydrogen peroxide (H_2O_2) is one of the families of reactive oxygen intermediates that are formed from the partial reduction of molecular oxygen. GPx catalyses the peroxides reduction and CAT takes care of H_2O_2 reduction. Because the activity of these enzymes was closely related one another, the balance between different antioxidant parameters is essential to the maintenance of cellular resistance against oxidants components (fig. 1). Considering this

statement, we calculated CAT/SOD and GPx/SOD ratios (table 2).

In the group of patients with ARMD and association ARMD and, cataract, the SOD activity is significantly decreased compared with the control.

Erythrocyte catalase, the first line of defence against hydroperoxides, in the group of patients with ARMD, cataract, and association ARMD and cataract was significantly decrease compared to the control group (tables 3, 4).

Table 2. The ratio values of CAT/SOD and GPx/SOD

	CAT/SOD	GPx/SOD
Control group	113.95	0.231
ARMD group	96.00	0.231
CATARACT group	102.49	0.199
ARMD and CATARACT group	119.28	0.207

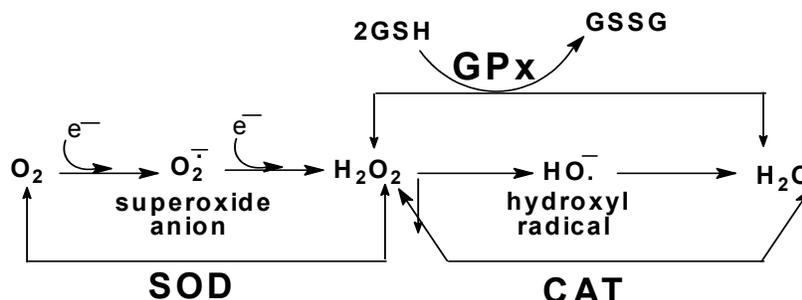


Fig. 1 The activity of antioxidant enzymes

Table 3. Erythrocyte CAT activity (K_{15} / g Hb) in the cases groups compared to control

	Control group	ARMD group	CATARACT group	ARMD and CATARACT group
Mean value \pm SD	811.3 \pm 27.25	536.22 \pm 48.78	692.87 \pm 38.45	696.65 \pm 34.71
95% CI	(801.56-821.01)	(533.82 – 538.61)	(679.09 – 706.64)	(685.14 – 708.16)
P		\leq 0.01	\leq 0.01	\leq 0.01

Table 4. Erythrocyte GPx activity (IU/ g Hb) in the cases groups compared to control

	Control group	ARMD group	CATARACT group	ARMD and CATARACT group
Mean value \pm SD	1.65 \pm 0.06	1.29 \pm 0.21	1.35 \pm 0.18	1.21 \pm 0.18
95% CI	(1.62 – 1.67)	(1.22 – 1.35)	(1.29 – 1.40)	(1.15 – 1.27)
p		\leq 0.01	\leq 0.01	\leq 0.01

In the group of patients with ARMD, and both ARMD and cataract, the higher reactive oxygen species could inactivate the antioxidant enzymes: superoxide anion O_2^- inhibited all three enzymes, H_2O_2 inactivated catalase and glutathione peroxidase. In order to

maintain an antioxidant status, their activity should be coordinated and sustained. Amstad and co have demonstrated on cell cultures that an increase only of SOD activity has toxic effects through H_2O_2 hyperproduction (9).

For the patients diagnosed with ARMD and cataract, GPx activity was significantly decreased compared with the control group.

The reactive oxygen species were higher in the group of patients with ARMD, ARMD and cataract groups. Superoxide anion inhibits all three enzymes and generates a hyperproduction of H₂O₂. The activity of the three enzymes should be sustained and coordinated for maintaining an antioxidant status.

The activity of SOD should be accompanied by a catalase and/or glutathione peroxidase activity.

Therefore in order to reveal the enzymatic antioxidant parameters, the CAT/SOD and GPx/SOD ratios are more significant (11,12).

The modifications of the values of CAT/ SOD ratio in the patients with ARMD (96 compared to 113.95 for the control group), have demonstrated

that only increase of SOD activity has toxic effects probably through H₂O₂ hyperproduction. A parallel increase of SOD and GPx (the higher ratio of GPx/SOD=0.231) of the same patients with ARMD, similar with the control group will determine a high resistance to a strong oxidant agent. The values of CAT/SOD of 102.49 and 119.28 respectively in the patients with cataract and both ARMD and cataract, demonstrated a low increase of SOD activity (113.95 in the control group).

Decreased values of SOD in our patients diagnosed with ARMD were registered in 100% of cases compared to 40% of cases in control group. SOD was decreased in 80% cases in the group of patients with cataract and 88.57% cases in the group with both ARMD and cataract compared to 40% in the control group (fig. 2).

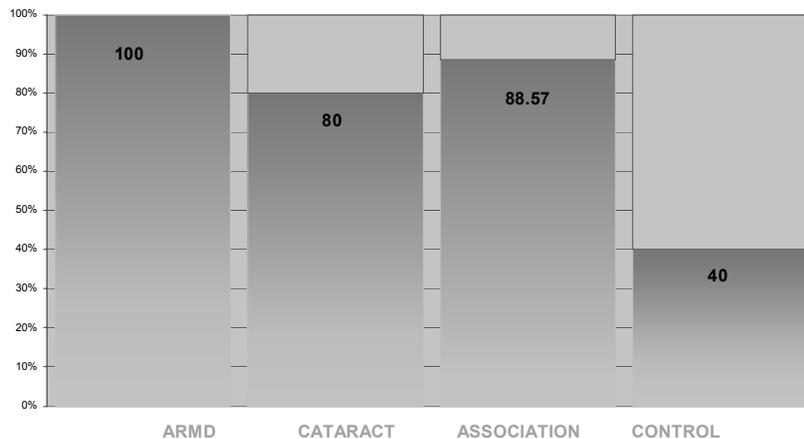


Fig. 2 The proportion (%) of patients with decreased SOD values by study groups

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Decreased values for catalase were found in 100% of cases for all the ophtalmological diseases, ARMD, cataract, and ARMD and cataract compared to 13.33% cases in the

control group (fig. 3). The low level of catalase in all ophtalmological diseases, proved the importance of these enzyme in order to decompose H_2O_2 , less reactive oxygen specie.

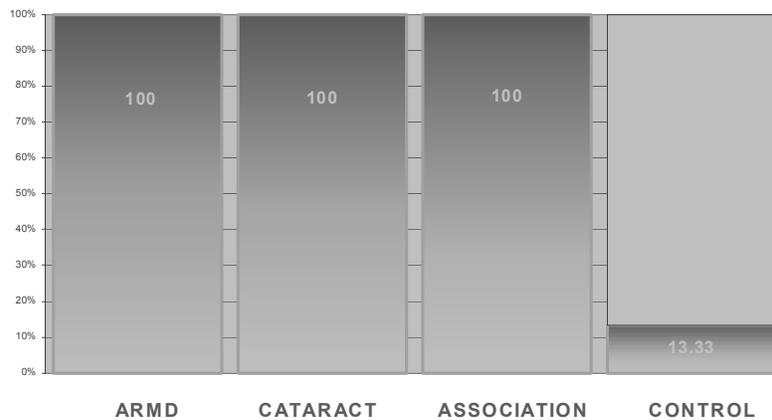


Fig. 3 The proportion (%) of patients with decreased CAT values by study groups

Decreased values of GPx were found in 100% of the cases diagnosed with ARMD, 87.5% of cases diagnosed with cataract, and 97.14% of cases diagnosed with both ARMD and cataract, compared to 60% of cases from the control group. GPx provides a second line of defences against hydroperoxides before them damage membranes and other cell components (fig. 4).

The values of CAT/SOD and GPx/SOD ratios were highly decreased in the decrease group versus the control group and have demonstrated an increase of SOD activity.

Our study revealed a significant association between the low level of antioxidant parameters and the high occurrence of ARMD.

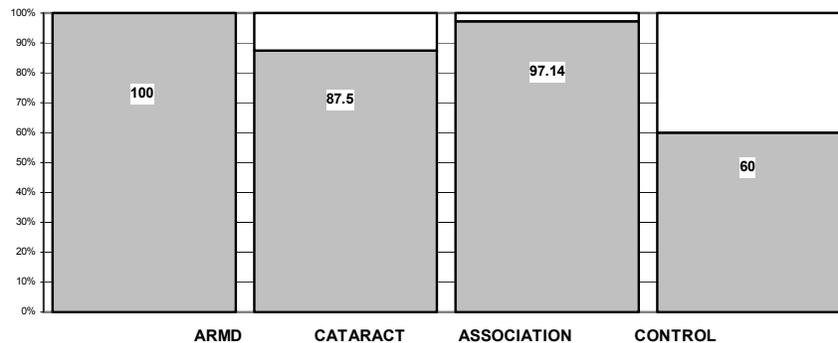


Fig. 4 The proportion (%) of patients with decreased GSHPx values by study groups

CONCLUSIONS

1. Decreased values of SOD were registered in the group of patient diagnosed with ARMD compared to the group of patient diagnosed with cataract and association ARMD and cataract (100% vs. 40%).
2. The level of catalase in all ophtalmological disease was significantly decreased.
3. Decreased values of GPx were found in all the cases with ARMD.
4. The values of CAT/SOD and GPx/SOD ratios show changes in the activity of enzymes.
5. Our study revealed a significant association between the occurrence of ARMD and the low level of antioxidant enzymes.

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