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Changes in blood antioxidants and several lipid peroxidation products in women with age-related macular degeneration

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PURPOSE. *The aim of this study was to evaluate the ferric reducing ability of plasma (FRAP), selected enzymatic and non-enzymatic components of the antioxidative system, and the intensity of peroxidative processes in the blood of patients with age-related macular degeneration (AMD).*

METHODS. *In the peripheral blood, we evaluated FRAP; concentrations of vitamins C, A, and E; and of thiols. We assayed the activity of enzymatic components of the antioxidative system-superoxide dismutase, catalase, ceruloplasmin and the concentration of reduced glutathione as an indicator of glutathione peroxidase activity. In order to determine the intensity of lipid peroxidation, we measured the concentrations of malondialdehyde and hydroxyalkenales (MDA-HNA) and conjugated diens (CD).*

RESULTS. *We found a significant increase in FRAP in patients with AMD compared with the control group. The average concentrations of vitamins A and C were low and vitamins E and GSH were significantly higher in AMD than in the control group. The activity of almost all the antioxidative enzymes was high. We found a significant increase in MDA-HNA but no difference in CD.*

CONCLUSIONS. *The significantly higher concentration of lipid peroxidation products in patients with AMD indicates an important pathogenic role of oxido-reduction disturbance. The high FRAP concentration may be one of the protective mechanisms in oxidation stress. The adaptive increase of the antioxidant barrier mostly involves the enzymatic components. (Eur J Ophthalmol 2003; 13: 281-6)*

KEY WORDS. *AMD, Antioxidants, Vitamins, Lipid peroxidation*

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INTRODUCTION

Age-related macular degeneration (AMD) is a primary degenerative disorder of the central retinal area with loss of visual acuity, most often seen in people over 50 years of age (1). AMD is divided into early and late forms. In the former, called age-related maculopathy (ARM), there are soft drusen and areas of hyper- or hypopigmentation. The latter form includes geographic atrophy (dry AMD) and a disciform variant (neovascular or wet), first described in 1885 by Haab as senile macular degeneration (2).

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AMD is the main cause of blindness in the elderly white population. It is estimated that a third of adults over 70 show signs of ARM, whereas the later stages (geographic atrophy or neovascular) are present in 2.8% of people over 65 and 7.2% of those over 75 (3, 4).

Recent articles have identified a low macular pigment level as a risk factor (5, 6). Other risk factors are genetic predisposition (7-10), cigarette smoking (11), postmenopausal estrogen decrease (12), cardiovascular diseases (13-15), and excessive exposure to sunlight (16).

In the last decade much attention has been focused on the connections between the concentrations of antioxidants and the presence of various disorders of old age. Since oxidative stress is important in the etiology of AMD antioxidants might have some protective effect (17-19). However, investigations so far have produced ambiguous or often contradictory results. Although the rods and cones are replaced every ten days their outer segments may be at particular risk of oxidative damage because of the high concentrations of polyunsaturated fatty acids in the photoreceptor outer segment membranes (20).

Because therapeutic possibilities are limited and the number of people with AMD is increasing, there is much interest in the pathogenesis and the risk factors of this disease and in finding effective protective agents.

There is increasing evidence that vitamins with antioxidative potential (e.g., vitamins A, C, and E) are protective for the vascular endothelium. They reduce inflammation and inhibit platelet aggregation, slowing the progression of atherosclerosis. The results of case-control studies concerning supplementation with antioxidative vitamins are often ambiguous but they generally indicate a decrease in the risk of degeneration and cardiovascular diseases, especially if vitamins are given early (21-23).

The antioxidant index of the body, including measuring the concentrations of erythrocyte enzymes and plasma non-enzymatic components, has been studied in patients with AMD by only a few researchers but the results were contradictory (19, 20).

We therefore investigated the ferric reducing ability of plasma (FRAP), selected enzymatic and non-enzymatic components of the antioxidative system, and the intensity of peroxidative processes in the blood of patients with AMD.

METHODS

This study enrolled 45 women, aged 55 to 71 years (mean age 65.1 ± 5.7) treated in our outpatient clinic for more than two years for AMD. Cases included 28 patients with ARM and 17 with more severe geographic degeneration. The control group consisted of 20 healthy women aged 50 to 65 years (mean 62.2 ± 5.2) with no ophthalmologic complications or family history of AMD. No patient was taking any antioxidant micronutrient supplement.

The eye examination included visual acuity, biomicroscopy, and ophthalmoscopy using a 90-diopter lens or direct ophthalmoscopy after dilation of the pupil. In all cases the diagnosis and classification of AMD were based on reduction of visual acuity to less than 5/5, ophthalmoscopy, and fluorescein angiography of the retina. ARM was diagnosed in eyes with clinically detectable pigment epithelial changes of hyper- or hypopigmentation and hard or soft drusen. Geographic atrophy was diagnosed in eyes with a sharply delineated, roughly round area of hypo- or depigmentation, at least one-third disc diameter in size, or an apparent absence of retinal pigment epithelium (RPE), with choroidal vessels more visible than in the surrounding areas (1). Disciform macular degeneration was found in eyes with serous or hemorrhagic detachment of the RPE or hemorrhage or a gray subretinal fibrous membrane involving the macula (1). Exclusion criteria were diseases other than AMD associated with neovascularization, retinal detachment, severe ocular trauma, pathologic high myopia, and intraocular inflammation.

In the morning (after 8 to 12 hours of fasting), blood specimens for biochemical assays were collected into tubes with 1.5 mg EDTA/mL of blood. Samples for ascorbic acid were deproteinized with 10% metaphosphoric acid, and all samples were then frozen at -70 °C.

We assayed the following in plasma:

1. FRAP, using the method described by Benzie and Strain (24);
2. Vitamin C, spectrophotometrically using ascorbate oxidase and the Fe(III)-batophenanthroline disulphonic acid system (25);
3. Vitamins A and E spectrophotometrically following Hansen and Warwick's method (26);
4. Thiols (free SH groups) following Wayner's modification of Koster et al method (27).

In order to determine the intensity of lipid peroxidation, we evaluated the following:

1. Malonyldialdehyde and hydroxyalkenales (MDA-HNA), spectrophotometrically using a LPO 586 Oxis Int. kit;
2. Conjugated diens (CD) based on the molar absorption coefficient = 21,000 x mol^{-1} x cm^{-1}.

In the hemolysate of the red blood cells (RBC), we assayed the following:

1. Superoxide dismutase (SOD) activity by kinetic spectrophotometry using a Randox standard kit;
2. Catalase activity according to Aebi (28);
3. Ceruloplasmin using its oxidative activity according to Schosinsky et al (29);
4. Reduced glutathione (GSH) using Ellmann's reagent (30).
5. Hemoglobin and total proteins in blood

Statistical analysis was carried out using the Statistica program with Student t-test for parameters with normal distribution or the Mann-Whitney test for those not normally distributed, assuming $p < 0.05$, $p < 0.001$, and $p < 0.0001$ as significant.

The project was carried out with the approval of The Bioethics Board of the Silesian University of Medicine (NN 013-653/I/99/2000). All subjects gave formal consent before participating in the study, and research followed the tenets of the Declaration of Helsinki.

RESULTS

The results of the biochemical investigations in the AMD and control patients are presented in Tables I, II, and III. The average concentration of vitamin A in AMD was markedly lower ($p < 0.0001$) than in the control group (Tab. I). We did not find a significant difference in the average level of vitamin C because of the large standard deviation in patients with AMD. The concentrations of vitamin E and of SH groups were markedly higher than controls (Tab. I).

FRAP was significantly higher ($p < 0.001$) in patients with AMD than the control group (Tab. II). We found increases in activity of all the enzymatic components of the antioxidative system. Ceruloplasmin and SOD were much higher ($p < 0.05$) in the AMD group than the control group. However, the concentration of GSH and catalase activity were similar in the two groups (Tab. II).

Concerning the redox status (Tab. III), we found a significant increase in MDA-HNA but no difference in CD , these being used as indicators of intensification of lipid peroxidation in patients with AMD.

DISCUSSION

It is difficult to establish reliable connections between the redox status and the development of AMD and other disorders of old age. The ambiguous results of the investigations to date are probably caused by the factors discussed below.

Firsty, the populations studied in the various investigations were different, with different nutritional habits. Then too, most studies measured the redox status as high, medium, or low, based on their own laboratory norms. Secondly, there is no commonly acceptable, standardized model stating the level of risk for AMD with any specific reduction in the concentrations of antioxidative substances. We too cannot establish the level of these substances in serum nec-

TABLE I - CONCENTRATIONS OF THE NON-ENZYMATIC COMPONENTS OF THE ANTIOXIDATIVE SYSTEM IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION (AMD) AND CONTROLS

	AMD (n = 45) mean ± standard deviation	**Control (n = 20) mean ± standard deviation**	**Student-t p (t-test)**	**Mann-Whitney p (u-test)**
Vitamin C, µmol/L	48.10 ± 22.56	57.30 ± 12.90	0.157374	
Vitamin A, µmol/L	105.32 ± 33.70	124.42 ± 26.58		<0.0001
Vitamin E, µmol/L	34.31 ± 8.15	25.84 ± 5.76	<0.0001	
SH, µmol/L	468.72 ± 108.31	390.4 ± 61.78	<0.05	

SH = Thiols

essary for protection against the progression of AMD. Finding a low concentration of antioxidants does not mean that antioxidative vitamin supplementation will reduce the risk of AMD.

One of the serious limitations in establishing the role of antioxidant factors in the development of senile degenerative disorders is the impossibility of measuring antioxidant concentrations in the retina of live patients. It must simply be assumed that the antioxidative vitamin concentrations in the blood serum and the activity of enzymes in the RBC correlate with their concentrations and activity in the eye.

We selected FRAP for study because it reflects the concentration of low molecular-weight non-enzymatic antioxidants and is independent - in contrast to the total antioxidant status - of the concentration of proteins in serum. FRAP permits a more objective evaluation of the nonprotein antioxidant activity. On account of financial considerations we could not measure the activity of glutathione peroxidase, and had instead only to define it by measuring reduced glutathione.

We analyzed FRAP because SOD, catalase, and other antioxidative enzymes, and the antioxidative vitamins, work synergistically against oxidative damage to body tissues. Thus, with high activity of one of the enzymes and low activity of the others, or with high concentrations of the antioxidative vitamins and lower activity of the antioxidative enzymes, the antioxidative system's defenses may be similar. In our study, the low concentrations of the antioxidative vitamins does not mean that total antioxidant activity is reduced, because of the simultaneous increase in activity of nearly all the enzymatic components of the system.

The body's antioxidative system must be considered as a whole and as a dynamic system. Antioxidative enzyme synthesis increases with intensive, lasting free radical synthesis. At the same time, non-enzymatic antioxidant concentrations may drop. In a sense, it is the organism's adaptive response to oxidative stress.

We found no published investigations of all these antioxidative elements together with an evaluation of

TABLE II - THE FERRIC REDUCING ABILITY OF PLASMA (FRAP) AND ACTIVITY OF THE ENZYMATIC COMPONENTS OF THE ANTIOXIDATIVE SYSTEM IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION (AMD) AND CONTROLS

	AMD (n = 45) mean ± standard deviation	Control (n = 20) mean ± standard deviation	Student-t p (t-test)	Mann-Whitney p (u-test)
FRAP, µmol/L	722.00 ± 105.12	621.25 ± 60.87	<0.001	
Reduced glutathione, µmol/g Hb	6.54 ± 0.66	6.33 ± 0.24	0.171085	
Superoxide dismutase, U/g Hb	1615.76 ± 93.12	1519.25 ± 103.85		<0.05
Catalase, U/g Hb	131.12 ± 12.12	126.86 ± 12.49	0.065456	
Ceruloplasmin, U/g protein	115.96 ± 21.96	98.15 ± 20.10	<0.05	

TABLE III - INDICATORS OF THE INTENSITY OF LIPID PEROXIDATION IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION (AMD) AND CONTROLS

	AMD (n = 45) mean ± standard deviation	Control (n = 20) mean ± standard deviation	Student-t p (t-test)
MDA-HNA, µmol/L	3.98 ± 1.21	3.25 ± 0.52	<0.001
CD, µmol/L	104.25 ± 39.06	121.80 ± 31.37	0.15456

MDA-HNA = Malondialdehyde and hydroxyalkenales, CD = Conjugated diens

the products of lipid peroxidation. Our results are in agreement with other findings (19, 20) indicating that a high antioxidative index is linked with a higher risk of developing the late neovascular form of AMD.

The results of the current study are different from those presented by other researchers (14, 18), who found no correlation between the antioxidative status of the parameters selected and the occurrence and development of AMD. This was the result- we believe-of the incomplete evaluation of the antioxidative system. In a group of 167 patients with varying levels of AMD, Mares-Perlman et al (31) found no significant difference in the carotenoid concentration in relation to the control group. The average vitamin E concentration was lower in patients with disciform degeneration, but there was no difference when it was measured in relation to total serum cholesterol. In a later work, these investigators set out to find out whether damage to the retina could be influenced by the presence of antioxidants and microelements, especially zinc, in the diet (17). They showed a protective influence of zinc on the development of ARM and found that the risk was lower in people taking vitamins C and E. However, the protective effect of minerals and antioxidative vitamins, also noted by other researchers (19), remains to be proved.

There are two theories about the protection against AMD by appropriate dietary measures: a proper diet can act against either the development of arteriosclerosis, which might be a cause of AMD, or the oxidation processes of the body through antioxidant factors.

The link between diet and antioxidant factor concentrations and the development of senile degeneration needs further examination. The significantly higher concentrations of lipid peroxidation products in patients with AMD indicates an important role of oxido-reduction disturbance in the pathogenesis of AMD. The high FRAP concentration may be one of the adaptive mechanisms for oxidation stress. The adaptive increase of the antioxidant barrier especially involves the enzymatic components of this system; however, a holistic examination of the oxido-reduction balance seems to be important.

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