Oxidative stress intensity in lens and aqueous depending on age-related cataract type and brunescence

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INTRODUCTION

Age-related cataract is a disease whose impact on society and the economy will increase as the population ages. Increasing age is the most consistent risk factor. However, every older person does not develop cataract, nor does everyone develop the same form. Oxygen is essential for aerobic life on Earth, as is the sun for diurnal species. But both of them have a damaging potential.

INTRODUCTION

Formation of harmful oxygen reactive radicals and oxidative stress are inevitable consequences of aerobic life. Besides all benefits of solar radiation for development of numerous life forms on Earth and development of the sense of sight, it has the capacity to initiate oxidative stress and cause photodynamic biomolecular changes.

Lens is exposed to ultraviolet (UV) rays that are not captured by cornea, and it absorbs them. By protecting retinal and other deep ocular tissues from those rays, it suffers damage. Oxida-
tive stress initiated by electromagnetic radiation of the UV part of the sun spectrum, a photo-oxidative stress, has been accused of cataractogenesis (1).

The contemporary hypothesis considers oxidative stress as the main event in age-related processes in the body, as well as in age-related cataract formation. Disturbance of balance between oxidative damages and antioxidative defense causes the oxidative stress. It damages proteins, enzymes, lipids, polysaccharides, and nucleic acids. It has a cumulative nature, and the level of assembled damaged biomolecules that could not have been repaired rises during the lifetime. Some events occurring during cataract genesis may resemble those during senescence of lens, but they are more intensive and extensive in nature (2, 3).

The lens poses defense against oxidative, i.e., photo-oxidative stress. It is manifested by low oxygen pressure in lens cells, slow rate of their metabolism, high concentration of vitamin C, glutathione, and sulfhydryl groups of crystallines, as well as by activity of other antioxidant vitamins and enzymes (4-7).

Aqueous humor is essential for the lens average metabolism. It supplies lens with energy necessary for synthetic, transport, and other processes. Besides glucose, aqueous contains amino acids for the protein synthesis, glutathione, vitamins, and hormones which are needed for lens metabolism and transparency maintenance, but it can influence negatively the processes inside the lens by its constituents (oxidative products and mediators of inflammation during uveitis, for example). However, the content of aqueous humor reflects both lens metabolism and its changes. Lactate in aqueous, for example, derives from lens anaerobic glucose metabolism. Hydrogen peroxide is an average constituent of aqueous humor, but its concentration is significantly different in healthy subjects and patients with cataract (8).

Human age-related cataract can be of cortical, nuclear, posterior subcapsular, or mixed origin (9). The nature of pigment in some cataract forms is a matter of investigation (10-12), and has been mostly linked to the tryptophane oxidation products. Etiopathogenesis of various forms of cataract could be different. It is not only a matter of the possible recognized risk factors for cataractogenesis (13-16, 24), but also of its different mechanisms and intensities of systemic and local oxidative stress, that, conversely, could be result of the same risk factors.

Contemporary cataract surgery methods reduce available material for such investigations, but they do not diminish general interest in this subject.

METHODS

This was a retrospective cross-sectional review of 80 analyzed samples of aqueous humor and lens corticonuclear blocks, obtained by extracapsular cataract extraction (ECCE) in cataract patients. Types of cataract were estimated during ophthalmologic examination, and confirmed during its extraction. Average clinical grading system of the cataract severity was used. All patients were operated in narcoleptic analgesia and local Xylocaine anesthesia. Mydriasis was induced preoperatively by Neo-Synephrine and atropine sulfas. Aqueous was taken by anterior chamber paracentesis in the beginning of operation. As classical ECCE was planned, corticonuclear blocks of lenses were taken for analytical purposes. Immediately after sample acquisition, samples were closed in individual capsules and deeply frozen.

Aqueous samples were analyzed by the method of estimation of antioxidant activity in serum and other liquids, and expressed as percent of Fe-induced malondialdehyde (%iMDA) (17), by using 0.1 mL of aqueous. Lenses were homogenized in TRIS-HCL buffer. In one part, total sulfhydryl groups (TSH) were estimated by Ellman’s reagent (18), without previous deproteinization of samples. In that manner, all sulfhydryl groups that could participate in antioxidative protection were estimated: those belonging to peptides (glutathione-GSH), and those bound to the proteins. In another, centrifuged part of homogenized lens tissue, after addition of three chlorine acetate, lipid peroxides (LP) were estimated by Fe-xylenol orange essay (19).

Data were analyzed by univariate analysis of variance (ANOVA), t test, Pearson correlation coefficient, and Spearman rank correlation coefficient.

RESULTS

We analyzed 80 samples of the lens corticonuclear blocks and aqueous humor obtained from 72 patients with age-related cataract. Eight of them had bilateral ECCE. There were 46 men and 26 women, age range from 45 to 84 (mean age 64.94±8.81).

In the group were 50 cortical, 16 mixed, and 14 nuclear cataracts. Number of pigmented, brunescent cataracts was 32. In the group with cortical type of cataract, 18 were incipient, 16 intumescent, and 16 mature. In the group with mixed type of cataract there were cortico-nuclear and cortico-nuclear and posterior subcapsular mixed forms, in different progression stages, but there were no cataracts. There were no pa-
tients with sole posterior subcapsular opacities in the cataract group. Antioxidative capacity expressed as percent of Fe induced malondialdehyde was measured in aqueous. Data were analyzed according to the cataract type and pigmentation. Average value of %iMDA in the whole group was 39.9±17.58% (range of variation 5.0–74.0%). The lowest value of %iMDA was found in the group of mixed type of cataract (16.69±7.07), and the highest in the group of nuclear cataract (45.17±16.1) (Fig. 1). In the cortical cataract type group it was 42.8±16.75. Variance analysis showed that mixed type of cataracts have a statistically lower values of antioxidative defense in aqueous humor, compared with two other types of cataract (F=10.77; p<0.001).

According to the color, brunescent cataracts had statistically lower values of %iMDA (t=2.89; p<0.001) (Tab. I). Maturation of the cortical cataract was related to the %iMDA. Maturity and %iMDA had a significant negative correlation (R=–0.305; p=0.026) (Fig. 2).

Values of total, protein, and non-protein sulphydryl groups—TSH in the lens corticonuclear blocks were 4.14±1.59 µM/g of tissue. Range of variation was between 1.5 and 8.8 µM/g. Mixed cataract type had the lowest values of TSH (2.35±0.46), compared with nuclear (4.44±1.46) and cortical cataract type (4.45±1.55), and that difference was significant (F=10.03; p<0.001) (Fig. 3). Comparing the concentration of TSH in pigmented and nonpigmented cataracts, a significant difference was established in favor to the nonpigmented ones (t=3.54; p<0.001) (Tab. I). Related to the maturity of the cortical cataracts, there was no correlation to the TSH (Spearman R=–0.098; p=0.488).

Intensity of lipid peroxidation also varied in different cataract types. Mean value of LP in corticonuclear blocks was 57.22±10.63 pM/mg of tissue, with variation between 33.1 and 80.7 pM/mg. The highest level of LP was found in the mixed type of cataract (69.94±5.23), then in the cortical type (55.29±10.05), and finally in the nuclear cataract type (54.24±10.14) (Fig. 3). Difference of concentrations of LP in the mixed cataracts compared to the two other groups was on the level of p<0.001, F=11.97. In the group of brunescent cataract values of LP concentration were significantly higher (t=3.28; p<0.001) (Tab. I). Correlation between LP and maturity of cataract was not found (Spearman R=0.150; p=0.289). Correlations among all three investigated parameters of oxidative stress, one to another individually, were on the level of
Cataract type, pigmentation, and oxidative stress intensity

Irreversible cell proteins and membrane lipids modification in cataractous lenses is initiated in the epithelial layer by oxidative stress stemming both from photodynamic reactions and from peroxides generated in ascorbate oxidation (21). The process spreads to the other lens layers as well. Values of antioxidant capacity in aqueous humor, measured as %iMDA, as well as lens sulfhydryl groups, which represent an important part of the lens antioxidant protection, were lowest in the mixed type of cataract and pigmented cataracts. Results point to higher intensity of oxidative stress and there is consistency with the findings of stronger lipid peroxidation in the same cataract type. Low level of antioxidant protection of aqueous here expresses oxidative damages in lens as the secondary event for aqueous, rather than primary event in that compartment. Moreover, a high level of correlation between LP in lenses and %iMDA in aqueous might confirm that (20).

Induced percent of malondialdehyde (%iMDA) could serve as a good parameter for estimation of total antioxidative protection in liquids. Its value is primarily in relation to the lipids that had not oxidized in vivo, and in physiologic and biochemical sense it responds to the total antioxidative protection. Although the level of cortical cataract maturation has a low clinical importance, we used it for investigative purposes, because, in our conditions, it was the cataract type where we could follow level of oxidative stress and compare it with the cataract progression. Values of %iMDA had statistically significant negative correlation to the cataract maturity. That indicates the higher consumption or low level of antioxidative factors, and higher intensity of the oxidative events with cataract development. But there is lack of correlation with TSH and LP concentrations.

A possible explanation could be in different models proposed in different cataract types genesis (22). Oxidation of sulfhydryl groups, cross-linking of proteins by disulfide connection, and further formation of high molecular aggregates are the events that characteristically happen during nuclear cataract formation, rather than during the cortical one. Ionic imbalance caused by loss of Na+K+ATPase function and water content increase is the proposed mechanism for cortical cataract genesis. Low values of %iMDA in more mature cataracts probably reflect antioxidant consumption in an attempt at lens transparency preservation.

Disulfide concentration increases in insoluble protein fraction with pigmentation and level of nuclear involvement in cataract genesis (22, 23). In developed nuclear and total cataract, concentration of disulfide bonds in favor of sulfhydryl is near 90% or more, and 16% only in moderate, sole posterior subcapsular cataract (25).

The level of reduced glutathione (GSH) decreases with the cataract progression. But its oxidized form (GSSG) increases mostly in nuclear, and less in cortical cataract. There are also evident changes in activity of enzymes that regulate GSH metabolism (26). In a transparent human lens only about 10% of sulfhydryl groups belong to the glutathione (non-protein groups). Carriers of the most part of sulfhydryl groups are lens proteins (24-26). They have significant antioxidative potential. With advancing age, in the center of the lens there might be no significant protein oxidation, even past age 80 (26).

It is well known that, during cataract genesis, intensive formation of disulfide bridges occurs between proteins, as between cysteine and methionine residues, and proteins and GSH (25, 26). Those formations seem to be less extensive in cortical cataract type. However, they are much

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**DISCUSSION**

**TABLE I - OXIDATIVE STRESS VALUES ACCORDING TO CATARACT BRUNESCENCE**

<table>
<thead>
<tr>
<th>Pigmentation</th>
<th>%iMDA*</th>
<th>TSH*</th>
<th>LP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brunescent</td>
<td>31.29±16.66</td>
<td>3.19±1.21</td>
<td>63.1±10.24</td>
</tr>
<tr>
<td>Nonpigmented</td>
<td>43.62±16.92</td>
<td>4.53±1.59</td>
<td>54.73±9.97</td>
</tr>
</tbody>
</table>

*p<0.001 (r=0.76 for %iMDA and TSH, r=–0.88 for LP and TSH, and r=–0.90 for LP and %iMDA) (20).
more pronounced in pigmented cataracts (11, 12, 23, 26). Mixed cataract type in this article corresponds mostly to the cortico-nuclear type, but they are not the total. It is possible that oxidative stress is more intensive in this type of cataracts, as it happens in more than one lens layer at the same time, indicating a higher level of oxidative stress. Cortical cataract maturity has no correlation with TSH concentration in this article. It could be related to the mechanism of different type of cataractogenesis that is explained above. Nuclear cataracts in this article have almost the lowest values of oxidative stress parameters. This could be related to different intensity of opacities that were detected in investigated cataract types, as was mentioned previously. However, it could be related to numerous and different mechanisms of oxidative stress. Oxidative stress is extremely complex in both directions—oxidative damage and antioxidative protection. It is possible that lipid oxidation and formation of lipid peroxides is one of the initial events in cataractogenesis (27). Hydrogen peroxide has the main pro-oxidative influence on lipid peroxide formation. However, increase of lipid peroxides could be of secondary value, because it reflects decrease in antioxidative protection level (28). The highest level of lipid peroxidation was detected in cataractous lenses of diabetic patients (29, 30). Yet the most frequent cataract type in diabetic patients is cortical, and thereafter posterior subcapsular (31). They can be pigmented as well. We have already investigated lipid peroxidation in diabetic patients with cataract (30), but values of their oxidative stress intensity have not been as high as in mixed type of cataract. High values of lipid peroxides inside the lens point to the same conclusion about high intensity of oxidative stress in the cataractogenesis. Mixed type of cataract and pigmentation are related to significantly higher level of oxidative damages in lipid fraction of the lens constituents. Recent reports found that mixed opacities in cataract formation could be significant predictors of 2-year mortality, independent of other risk factors (32). Yet the true meaning of these and other results should be further evaluated. Mixed cataracts with nuclear/posterior subcapsular component have shown significant association with higher risk of death, particularly mortality for malignancy (33). Other study has shown increased 4-year mortality in subjects with mixed or any nuclear opacities, while coexisting diabetes further increases that rate in cataract patients (34). Brunescent cataract is associated with the presence of chromophores with light-absorbing and fluorescent properties. Some of them have origin in photo-oxidation of tryptophan, or in advanced glycation products (11, 12, 35, 36). The nature of pigment in brunescent cataracts, as well as in clear lenses during senescence, has been a matter of investigation. It is possible that the source of color changes in cataractous lenses should be sought in its lipid fraction, as well as in the protein one.

CONCLUSIONS

As this is a retrospective transversal study, it shows the present state of some of the main cataract types development only, and that limits more extensive conclusions about cataractogenesis. In this study we used lens corticonuclear blocks and not the whole lens or its defined parts. In that way, after homogenization of material, we mostly analyzed content of nuclear and cortical fibers. The results confirm the role of oxidative stress in age-related cataractogenesis and show that importance or mechanism of oxidative stress in genesis of different types of cataract could not be the same for all of them. The lack of correlation between cortical cataract maturity and TSH and LP concentrations deserves further investigation. The same conclusion is related to the high level of lipid peroxides in pigmented cataracts, which may point to the different nature of pigment source in cataract than proteins alone. Subjects with mixed lens opacities should be observed further, as the presence of mixed cataract may point to some changes on the level of the body.

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