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Antioxidant content and cytological examination of aqueous fluid from patients with age-related cataracts at different stages

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ABSTRACT. We investigated the antioxidant content and conducted a cytological examination of the aqueous fluid and lenses of patients with age-related cataracts at different stages. The levels of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) in the aqueous fluid and lenses were determined by the xanthine oxidase method, the colorimetric method, and the improved reduced glutathione (GSH) depletion method, respectively. SOD, CAT, and GSH-PX content in the aqueous fluid and lenses decreased significantly with increasing lenticular nucleus hardness grading. However, the number of white blood cells, neutrophils, monocytes, lymphocytes, and eosinophils did not vary significantly with varying lenticular nucleus hardness. Antioxidant content examination is an important quantitative indicator for clinical diagnosis and treatment of age-related cataracts. Antioxidant content in the aqueous fluid and lenses decreased

significantly with increasing lenticular nucleus hardness grading. Lenses at hardness level V had the lowest content of antioxidants.

Key words: Age-related; Cataracts; Antioxidants; Cytology

INTRODUCTION

Age-related cataracts is an eye disease caused by multiple factors. It seriously affects visual function and accounts for more blindness than any other eye disease. At present, most researchers generally believe that oxidative damage by free radicals is an important factor for age-related cataracts formation (Choy et al., 2011). Here, we focused on the antioxidant content and the results of a cytological examination of the aqueous humor of age-related cataracts patients at different stages.

MATERIAL AND METHODS

Materials

One hundred subjects (53 males and 47 females) were enrolled. The mean age of subjects was 68.1 ± 1.3 years (51-86 years). All patients were diagnosed with age-related cataracts. Among them, the level of the lenticular nucleus hardness was II in 23 cases, III in 36 cases, IV in 38 cases, and V in 3 cases. No patients had ocular trauma or high myopia history, etc. Aqueous fluid (0.2 mL) was extracted using a 1-mL syringe and preserved at 4°C. Intracapsular lens extraction was performed and the lens was washed with phosphate-buffered saline. After drying on filter paper, the lens was ground into a homogenate while cooling using an ice bath. Finally, the homogenate was centrifuged at 10,000 *g* for 15 min and examined after 12 h.

Instruments and equipment

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) kits were bought from Dong Song bo Industry Biotechnology (Beijing, China). An Axiostar Plus microscope was acquired from Zeiss (Oberkochen, Germany).

Methods

SOD content was determined by the xanthine oxidase method. Homogenate (0.003 mL) was mixed with 0.1 mL H_2O_2, 0.1 mL liquid matrix, 0.1 mL nitroso agent, and 0.2 mL enzyme solution, and incubated at 37°C for 40 min. The mixture was then added to 2 mL color developing agent and examined at 550 nm wavelength. SOD content = (absorbance of control tube-absorbance of test tube) ÷ absorbance of control tube ÷ 0.5 x dilution ratio.

GSH-PX content was determined by the improved reduced GSH depletion method. The homogenate (0.02 mL) was diluted with 1 mL water and incubated at 37°C for 5 min. The solution (0.4 mL) was added to 4 mL agent and centrifuged at 3500-4000 rpm. The GSH-PX content was detected at 420 nm and calculated by colorimeter with a 1 cm optical path. GSH-PX = ($OD_{control}$ -OD_{test}) x A x 5 ÷ 3 min x protein weight. A = standard GSH concentration/ optical density (OD) standard GSH absorbance.

The CAT level was determined by the colorimetric method. Three tubes were used as blanks. In detail, blank 1 contained 1 mL liquid matrix, 1 mL ammonium molybdate and 0.2 mL

homogenate; blank 2 comprised 1 mL liquid matrix, 1 mL ammonium molybdate, 0.2 mL buffer; and blank 3 included 1.2 mL buffer and 1 mL ammonium molybdate. The homogenate (0.02 mL) was diluted to 1 mL and incubated at 37°C for 1 min to prepare the test tube. After mixing with 1 mL liquid matrix, 1 mL ammonium molybdate was added and the investigation was conducted at 405 nm after 10 min. CAT (kU/L) = $(OD_{blank\ 1}-OD_{test})/(OD_{blank\ 2}-OD_{blank\ 3})$ x 325.

White blood cells were counted using a counting plate and a Zeiss Axiostar Plus microscope. The aqueous fluid was centrifuged at 1500 rpm for 10 min and colored for counting.

Lenticular nucleus hardness was mainly determined by slit lamp examination based on color for grading: Level I, the lenticular nucleus was transparent and soft; Level II, the lenticular nucleus presented as white or yellowish-white; Level III, the middle nucleus presented as yellow; Level IV, there was a hard core with an amber nucleus; and Level IV, there was a special hard core with a brown or black nucleus.

Statistical analysis

Measurement data are reported as means and standard deviation (means ± SD). Differences between groups were analyzed using the F-test or the chi-square test. All statistical analyses were performed using the SPSS19.0 software (Chicago, IL, USA). P values < 0.05 were considered to be statistically significant.

RESULTS

SOD, CAT, and GSH-PX content in the aqueous fluid and in lenses of different lenticular nucleus hardness were examined. The content of SOD, CAT, and GSH-PX decreased significantly with increasing lenticular nucleus hardness (P < 0.05) (Table 1).

Table 1. SOD, CAT, and GSH-PX content in aqueous fluid and lenses of varying lenticular nucleus hardness.

Lenticular nucleus hardness	SOD (U/mL)	GSH-PX (U/mL)	CAT (U/mL)
Level II	95.46 ± 11.08	83.62 ± 22.47	0.18 ± 0.12
Level III	86.72 ± 13.4	69.36 ± 21.52	0.11 ± 0.08
Level IV	76.68 ± 11.21	52.16 ± 19.10	0.08 ± 0.12
Level V	61.35 ± 10.30	40.03 ± 22.18	0.05 ± 0.03
F	6.52	5.73	6.12
P	<0.001	<0.01	<0.01

P: comparison among groups.

Aqueous fluid cytological examinations were conducted for lenticular nuclei of varying hardness. The number of white blood cells, neutrophils, monocytes, lymphocytes, and eosinophils did not vary significantly with varying lenticular nucleus hardness (Table 2).

Table 2. Aqueous fluid cytology test with varying lenticular nucleus hardness.

Lenticular nucleus hardness	Neutrophils (cell count/μL)	Monocytes (cell count/μL)	Lymphocytes (cell count/μL)	Eosinophils (cell count/μL)	White blood cells (cell count/μL)
Level II	8.1 ± 2.2	5.1 ± 1.7	0.3 ± 0.1	3.5 ± 2.0	50.2 ± 11.2
Level III	8.5 ± 3.1	5.5 ± 1.2	0.2 ± 0.1	3.1 ± 2.1	53.1 ± 13.1
Level IV	8.8 ± 2.5	4.9 ± 1.8	0.1 ± 0.1	3.3 ± 2.6	52.2 ± 11.6
Level V	8.1 ± 2.9	5.2 ± 1.3	0.3 ± 0.2	3.2 ± 2.3	51.5 ± 10.8
F	0.22	1.40	1.20	1.35	1.56
P	>0.12	>0.07	>0.07	>0.07	>0.12

P: comparison among groups.

DISCUSSION

Cataracts have become a hot topic for society following medical developments and the general extension of human longevity (Esfandiari et al., 2008; Hafizuddin et al., 2011).

Li and Spector (1996) proposed that oxidative damage is the active factor in the formation of cataracts. GSH is reduced while mixed disulfide comprising GSSG and protein increases in lenses following oxidative damage (Frankel and Berenbaum, 2006; Hosu et al., 2011a). A series of changes in elderly patients' lenses appeared, such as pigmentation, reduced transparency, and protein structure change, all of which make the lens more susceptible to oxidative damage (Rautenbach et al., 2010; Hosu et al., 2011).

SOD is an important antioxidant enzyme in the body (Esfandiari et al., 2008). SOD, GSH-PX, and CAT decreased in the aqueous fluid and lenses of the patients with age-related cataracts with increasing hardness of the lens nucleus. Large amounts of reactive oxygen species accumulate in the aqueous fluid and lenses with age; they cause SOD and GSH-PX consumption, changing the soluble proteins in the lens into insoluble proteins, which results in lens opacity (Lu and Rasco, 2012; Gupta and Ahmad, 2013). Reduced CAT weakens the antioxidant capacity of the lens epithelial cells and induces their apoptosis, which also causes cataracts (Ravipati et al., 2012; Zhu et al., 2013).

A lack of significant changes in the number of white blood cell, neutrophils, monocytes, lymphocytes, and eosinophils with varying lenticular nucleus hardness indicates that age-related cataracts have no relationship to eye inflammation (Croft et al., 1995; Sakr and El-Metwally, 2009).

In conclusion, aqueous fluid antioxidant content detection is an important quantitative indicator for the clinical diagnosis and treatment of age-related cataracts. Aqueous fluid antioxidants decrease significantly with increasing lenticular nucleus hardness, and reach a minimum at hardness Level V, at which point an ultrasonic emulsification operation is unsuitable (Bonaccio et al., 2013; Zhu et al., 2013).

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