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Malondialdehyde level in the lens of complicated cataract patients with the instillation of diclofenac sodium 0,1% eye drop pre-operatively

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ABSTRACT

Wiwik Widowati, Suhardjo, Wasisdi Gunawan, Retno Ekantini - ***Malondialdehyde level in the lens of complicated cataract patients with the instillation of diclofenac sodium 0,1% eye drop pre-operatively***

Objective: To determine the level of malonaldehyde of lens in complicated cataract patients receiving instillation of diclofenac sodium 0,1% eye drop, 3 times daily, 2 weeks before undergoing surgery.
Materials and methods: A cross sectional study on complicated cataract patients who underwent extracapsular cataract extraction, was conducted at Dr. Sardjito General Hospital, dr. Yap Eye Hospital and mass cataract surgery in Yogyakarta and surrounding area, from November 2002 until November 2003. Subjects were consecutively recruited and classified into two groups. Twenty patients received diclofenac sodium 0,1% eye drop 3 times daily for two weeks before lens extraction, and 20 patients who received no treatment served as control. On the day of surgery, the lens was taken to measure the level of MDA at the Laboratory of Biochemistry. The difference of MDA level between the two groups was statistically analyzed by *student t test*.
Results: The mean MDA level in the group receiving diclofenac sodium was 4,386 ± 2,625 nmol/g, while in the group without diclofenac sodium it was 6,728 ± 3,422 nmol/g. This difference was statistically significant with p = 0.020.
Conclusion: The mean MDA level in diclofenac sodium group was lower than that without diclofenac sodium group.

Key words: malondialdehyde level - caractogenesis - complicated cataract - diclofenac sodium.

ABSTRAK

Wiwik Widowati, Suhardjo, Wasisdi Gunawan, Retno Ekantini - ***Kadar malondialdehid pada lensa katarak komplikata dengan pemberian tetes mata natrium diklofenak 0,1% sebelum operasi***

Tujuan: untuk menentukan kadar malondialdehid lensa katarak komplikata pada pasien yang mendapat tetes mata natrium diklofenak 0,1%,3 kali sehari selama 2 minggu sebelum dilakukan operasi.
Bahan dan cara: Suatu penelitian potong lintang yang dilakukan terhadap pasien katarak komplikata yang menjalani operasi ekstraksi katarak ekstrakapsular di RS Dr. Sardjito, RS. Mata Dr. Yap dan operasi katarak massal di Yogyakarta dan sekitarnya mulai November 2002 hingga November 2003. Subyek diambil secara berurutan dan dikelompokkan menjadi dua. Kelompok pertama terdiri dari 20 pasien yang mendapat tetes mata natrium diklofenak 0,1%, 3 kali sehari selama 2 minggu sebelum operasi, dan kelompok kedua terdiri atas 20 pasien tanpa pemberian tetes mata natrium diklofenak sebagai kontrol. Setelah dilakukan operasi katarak, lensa diambil untuk dilakukan pemeriksaan kadar malondialdehid di Laboratorium Biokimia. Perbedaan kadar MDA antar kedua kelompok dianalisis dengan *student t. test*.

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Hasil: Rerata kadar MDA pada kelompok yang mendapat tetes mata natrium diklofenak adalah 4,386 ± 2,625 nmol/g, sedangkan kelompok tanpa natrium diklofenak memiliki rerata 6,728 ± 3,422 nmol/g. Perbedaan ini secara statistik bermakna dengan nilai p = 0,020.
Simpulan: Rerata MDA pada kelompok natrium diklofenak lebih rendah daripada kelompok tanpa natrium diklofenak.

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INTRODUCTION

Cataract is a major cause of blindness in Indonesia and in the world as well. The prevalence of blindness in Indonesia is 1.5%, which may be resulted from refractive (9.5%), corneal (6.4%), lens (52.0%), ocular nerve anomaly (13.4%), inflammation (2.4%), and others (1.8%)[1]. Incidence of blindness on account of cataract in the developing countries varies from 15% to 20%.[2]

Some local and systemic changes, such as oxidative stress, aging process, both radiation and chemical exposure as well as osmotic cataract, may account for opacity of crystaline lens.[3] The terminology of complicated cataract refers to cataract due to local eye disease, especially inflammatory disease which is markedly characterized by chronic uveitis; however, it may be a result of degenerative conditions such as chronic retinal detachment, glaucoma, and pigmentation retinitis. In addition, intraocular tumor, ocular ischemia, Raynaud's disease, obliterans tromboangitis, anterior segment necrosis, and retinal detachment surgery may account for complicated cataract. The most common etiology of complicated cataract, however, is iridocyclitis which is usually accompanied by posterior synechia.[4]

Inflammatory process results in the formation of prostaglandin including the one originated from endoperoxide. Free radicals originated from oxygen are released concomitantly with the formation of prostaglandin [5,6]. Therefore, inflammatory process is followed by increased free radicals.[7,8]

Oxidative stress occurs when the formation of reactive oxygen is faster than the ability of the cells to eliminate it through a mechanism involving dismutase superoxide, katalase, and glutation peroxidase enzyme. The adverse effect of reactive oxygen is thought to be the cause of several pathological anomalies of tissue including cataract. Peroxide lipid forms when a reaction between oxyradicals and unsaturated fatty acid, which is found in the cells as glycerilester, in phospholipid or triglyceride occurs. Oxidation of phospholipid membrane is thought to increase membrane cell permeability and or inhibit membrane ion pump. Absence of barrier function will lead to edema disturbance of electrolyte imbalance and increased intracellular calcium, thus resulting in disturbance of cell function.[9,10]

Not only does lipid peroxidation cause direct cell membrane damage, but also it evokes secondary impairment through aldehyde decomposition. Hydroperoxide lipid is unstable and can decompose into various aldehyde such as malondialdehyde (MDA) and 4-hydroxyalkenal. This kind of aldehyde may react fast as it meets protein and therefore inhibit its functions. Both lens and retina are sensitive to oxidative damage [9]. Malondialdehyde is the most reactive aldehyde. MDA and other short chains of aldehyde compound will result in intra and inter protein aggregation, so that molecular weight increases and crystalline lens clarity decreases.[3] Lian *et al.* reported that the level of MDA in senile and complicated cataract lens was significantly higher than that in the normal lens with higher MDA level in complicated cataract.[11]

High level of MDA is one of risk factors for cataract. The level of corneal, lens, ciliary body, and retinal MDA increases as intraocular inflammation occurs. Nonsteroid antiinflammatory drugs can reduce inflammatory process and consequently decrease the production of free radicals. NSAID has an effect to prevent free radicals formation.[12,13,14] Decreased free radicals will happen along with decreased lipid peroxidation production.

The objective of the study is to compare the level of MDA in complicated cataract lens receiving NSAID eye drop pre-operatively and that in complicated cataract lens without pre-operative NSAID.

MATERIALS AND METHOD

A cross sectional study was performed (prospectively) on complicated cataract patients at the Dr. Sardjito General Hospital, dr. Yap Eye Hospital, and mass cataract surgery in Yogyakarta and the surrounding areas from November 2002 until November 2003.

The subjects of the study were divided into two groups; 1st group consisted of complicated cataract patients receiving eye drop containing diclofenac sodium 0.1% three times daily for two weeks pre-operatively, 2nd group consisted of complicated cataract patients who did not receive eye drop. Sampling was performed consecutively.

The inclusion criteria of the subjects of the study were: 1) patients with complicated cataract due to uveitis, glaucoma, trauma, and retinal ablation, 2) non diabetic mellitus patients, 3) the patients did not develop myopia with more than 6 Dioptri, 4) those who did not consume drugs that could influence the level of MDA (alopurinol, Vitamine E, steroid, NSAID), 5) willingness to be included in the study. The patients with complicated cataract who were allergic to NSAID eye drop were excluded. The benefit of the study was explained to the patients with inclusion criteria and they were included in the study with informed consent.

The samples examined were complicated cataract lenses removed by extracapsular cataract extraction. The lenses were then put into a test tube with TCA solution 15% + C solution to stop the reaction. The MDA level of the lenses was examined in the Biochemical Laboratory of Medical Faculty of Gadjah Mada University. It was conducted by adding SDS 8.1% 200 µL, HCl (0.5 M) 1.5 ml, TBA 20 nM 1.5 ml, DDW 250 µL solutions to the lens samples. All of the solutions were mixed and heated up to 90°C for 15 minutes in waterbath. Then, it was chilled for 10 minutes. DDW 1 ml and normal butanol pyridine 5 ml were added; then it was centrifuged at 3000 rpm for 15 minutes. Supernatan was read by spectro-fluorometer at 520 nM wave length with 550 nM emition.[15]

Independent variables of the study were complicated cataract lenses receiving diclofenac sodium 0.1% eye drop or those which did not receive diclofenac sodium 0,1%. Dependent variables, on the other hand, were the levels of MDA in complicated cataract lenses. Confounding variables were age, smoking habit (>10 pieces/day), length of exposure to sunshine, consumption of liquor (>4 glasses/day).

The data were processed by computer (SPSS program). Student t test was performed to analyze the significant difference of mean age and MDA level in the lenses in the two groups. Chi square test was undertaken to analyze the difference of sexes, occupations, educations, sun exposure, smoking habit, cataract etiologies, and lens opacity in the two groups.[16]

RESULTS AND DISCUSSION

There were 40 lenses of complicated cataract patients fulfilled the inclusion criteria. The patients were divided into two groups, each of which consisted of 20 patients. Group A consisted of complicated cataract patients receiving diclofenac sodium 0.1% pre-operatively; Group B consisted of patients with complicated cataract who did not receive diclofenac sodium 0.1%. Demographical characteristics of complicated cataract patients are available in TABLE 1 and TABLE 2.

The mean age of group A was 57.70 ± 14.74 years, whereas that of group B was 59.20 ± 13.93 years. There were 15 male patients (75.0%) and female patients (25.0%) in group A; in group B there were 12 male patients (60.0%) and 8 female patients (40.0%).

The length of exposure to sunshine less than 7 hours/day was found 90% in group A and that of more than 7 hours/day was 10%, whereas that of less than 7 hours/day was found 75% in group B and that of more than 7 hours/day was 25%. Smoking habit less than 10 pieces/day was found 5% in group A and that of more than 10 pieces/day was 5%; in group B it was found 20% and 10% respectively. (TABLE 2).

Uveitis, trauma, retinal detachment, and glaucoma were the etiologies of cataract in the study. Lens characteristics and the etiology of complicated cataract patients are available in TABLE 3.

TABLE 1. Demographical characteristics of the complicated cataract patients

Variables	Group A	Group B	*p*
Age (Mean)	57.70±14.74	59.20±13.93	0.854
Sex			
Male	15 (75.0)	12(60.0)	0,311
Female	5 (25.0)	8 (40.0)	
Occupations			
Housewives	4 (20.0)	3 (15.0)	0.735
Labors	4 (20.0)	2 (10.0)	
Farmers	3 (15.0)	4 (20.0)	
Private employees	4 (20.0)	7 (35.0)	
Civil Servants	1 (5.0)	2 (10.0)	
Retired	4 (20.0)	2 (10.0)	
Educations			
Elementary School	6 (30.0)	6 (30.0)	0.783
Junior High School	4 (29.0)	6 (30.0)	
Senior High School	9 (45.0)	6 (30.0)	
College Degree	1 (5.0)	1 (5.0)	

TABLE 2. Length of sun exposure and smoking habit of the complicated cataract patients.

Variables	Group A	Group B	*p*
Sun exposure			
< 7 hours	18 (90.0)	15 (75.0)	0.204
> 7 hours	2 (10.0)	5 (25.0)	
Smoking habit			
Did not smoke	18 (90.0)	14 (70.0)	0.260
< 10 pieces/day	1 (5.0)	4 (20.0)	
> 10 pieces/day	1 (5.0)	2 (10.0)	

TABLE 3. Lens characteristics and the etiology of complicated cataract patients

Variables	Group A	Group B	*p*
Cataract etiologies			
Uveitis	13 (65.0)	6 (30.0)	0.112
Trauma	6 (30.0)	9 (45.0)	
Glaucoma	1 (5.0)	4 (20.0)	
Retinal detachment	0 (0)	1 (5.0)	
Lens opacity			
Grade I	4 (20.0)	0 (0)	0.108
Grade II	3 (15.0)	4 (20.0)	
Grade III	13 (65.0)	16 (80.0)	

Free radicals and lipid peroxidation product were produced in the anterior segment of experimental autoimmune uveitis[7]. Free radicals were morphologically and biochemically proved to be produced by uveorenitis and retinal damage was precipitated by lipid peroxidation in the retinal cell membrane [17]. The role of free radicals in the retinal detachment process was supported by the finding of malondialdehyde in the subretinal fluid of retinal detachment patients [18]. In the case of primary open angle glaucoma, ascorbic acid level of aqueous humor was proved to decrease and it, therefore, resulted in increased production of lipid peroxidation. Increased level of lipid peroxidation and stage III or IV glaucomatous patients were proved to be correlated each other; therefore, it was assumed

that open angle glaucoma was caused by lipid peroxidation as a result of inadequate ascorbic acid.[19]

Statistical analysis exhibited the distribution of subjects in the two groups by age, sex, occupation, education, sun exposure, smoking habit, cataract etiology, and degree of lens opacity were not statistically different ($p > 0.05$). Hence, the influence of variables on the results of the study in the two groups could be neglected (TABLE 1, TABLE 2 and TABLE 3).

The MDA level of myopic senile cataract lens, as reported by Micelli-Ferrari T., was 7.6±0.5 nmol/g.[20] In our study, the mean level of MDA of complicated cataract lenses which did not receive diclofenac sodium 0.1% was 6.728±3.42 nmol/g. The mean level of MDA of the two groups can be seen in TABLE 4.

TABLE 4. Mean level of MDA in the two groups

Group	n	Mean ± Standard Deviation	*p*
Group A	20	4.386±2.625	0.020
Group B	20	6.728±3.422	

The results of measurement of MDA level were in normal distribution; therefore, parametric analysis was performed by Student t test. Mean level of MDA of group A patients receiving diclofenac sodium 0.1% three times daily for two weeks pre-operatively was 4.386±2.635 nmol/g, whereas the one in group B patients without antiinflammatory eye drop pre-operatively was 6.728±3.422 nmol/g. By Student t test, the levels of MDA in the two groups were significantly different ($p = 0.020$). The level of MDA in complicated cataract lenses receiving diclofenac sodium 0.1% was lower than that without receive diclofenac sodium 0.1% pre-operatively.

Some non-steroid antiinflammatory drugs and agents containing antioxidant are known to affect the level of MDA in the lens. Rodriguez reported decreased concentration of lipid peroxidation in the retina and lens of rabbits after receiving silicone oil with phenylbutasone 80 μg/ml compared to silicone oil without phenylbutasone.[12] Awasthi reported that curcumine therapy would activate isozyme rGST8-8 in the epithel of lens of mice in vitro, so that rGST8-8 would bind 4-HNE, which is one of lipid peroxidation products. They, therefore, assumed that curcumine is an agent that can give protection against cataractogenesis induction by lipid peroxidation.[13] Triyono reported that myopic senile cataract patients receiving vitamine E 100 mg/day for one month exhibited significantly lower level of lens MDA (median 3.0 nmol/g) ($p= 0.03$) than those who received vitamine E 30 mg/day for one month (median 6.4 nmol/g).[21]

Mamchur *et al* reported that indomethacine, diclofenac sodium, and salysilic acid can alter the amount of MDA and the activities of superoxide dismutase (SOD) in the brain structure. These agents may accelerate the activities of SOD and thus lipid peroxidation is inhibited.[22]

Other studies investigating the effect of diclofenac sodium on MDA level in complicated cataract lenses have not been available. Yet, the differences of MDA level in the lens are probably due to diclofenac sodium capability in preventing free radical formation through a mechanism of decreasing inflammation[23] or this agent can accelerate SOD activities as reported by Mamchur *et al.*[22]

CONCLUSION

Complicated cataract patients receiving diclofenac sodium eye drop three times daily for two weeks pre-operatively exhibited significantly lower MDA level of lens than those who did not receive it pre-operatively ($p= 0.020$).

A further study to identify the mechanism of diclofenac sodium in decreasing the level of MDA in complicated cataract and its capability of preventing cataract formation whose etiology is a more specific complicated cataract is needed.

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