Evaluation of Redox Enzyme Superoxide Dismutase Activity Level in Aqueous Humor of Primary Glaucoma

Masitha Dewi Sari, Hera KW Siregar

Department of Ophthalmolgy, Medical Faculty Sumatera Utara University, Medan, Indonesia

***Abstract*- Background** : Glaucoma caused loss of visual function loss by damaging the optic nerve. Aqueous humor plays an important role in the pathophysiology of the eye. One of the function is to protect eyes from the ultraviolet rays and free radical. Oxidation and free radical and oxygen reactions can effect the activity cell of trabecular meshwork especially endothelial cell. Superoxide dismutases are enzymes involved in the protection against oxidative stress by detoxification of superoxide. The present study was design to determine level activity of SOD in aqueous humor of primary glaucoma patients

Methods :A case-control prospective study, we analysed aqueous humor 20 eyes (20 patients) primary glaucoma patients who underwent trabeculectomy. For control, aqueous humor of 26 age-matched senile cataract patients was collected. Activities of redox enzyme superoxide dismutase were measured by sphectrophotometrically.

Results : The activity level of SOD in aqueous humor was significantly lower compared to cataract patients ($p<0,05$).

Conclusion :These results suggest that lower redox enzyme is an important risk factor in the development of primary glaucoma. Assays of redox enzymes activities could provide marker to identify individuals predisposed to primary glaucoma.

***Index Terms*-** superoxide dismutase, aqueous humor, primary open angle glaucoma

I. INTRODUCTION

Glaucoma refers to a group of diseases that have common a characteristic progressive optic neuropathy with associated visual function loss and higher intraocular pressure. Glaucoma continues to be a major public health problem. Glaucoma is the second leading cause of blindness worldwide after cataracts including in Indonesia. This disease is typically asymptomatic until advanced visual field loss occurs[1,2,3]. Diagnosis of this neuropathy is based on optic disc change, visual field defects and intraocular pressure (IOP) elevation. The final common pathway for all potential etiologies of glaucoma is optic nerve damage. There are many postulated mechanisms of ganglion cell damage included raised IOP, genetic, vascular dysregulation, glutamate exitotoxicity, ocular ischemia, and oxidative stress are well established pathogenetic factor. Oxidative stress, in particular, seems to be a key player in glaucoma pathogenesis.[4,5,6]

Oxidative stress generally is induced through formation of multiple reactive oxygen species including hydrogen peroxide and superoxide that can initiate and propagate free radicals. The oxidative burden between prooxidant and antioxidant is oxidative stress that damage cellular and tissues macromolecules such as lipids, proteins and results in cellular.[6]

Many biologic substances growth as an oxidative marker such as superoxide dismutase. A number of studies in vitro and in vivo suggested that there was a role of oxidative stress marker in glaucoma patients. Flammer reported the destroyed of DNA in trabecular meshwork was higher in glaucoma patients compared to normal patients.[7] Antioxidant superoxide dismutase, catalase and gluthathione peroxide is an antioxidant and redox enzyme. Superoxide dismutase (SOD) is an enzyme that alternately catalyzes the dismutation of superoxide radical into either ordinary molecular oxygen or hydrogen peroxide. Superoxide is produced as a by-product of oxygen metabolism and if not regulated, causes many types of cell damages. SOD has been reported to have a protective role in cells and extracellular components from damges related to inflammatory process in the pathogenesis of many diseases. SOD is present in all eye tissues, mainly in the cytosol of differentiated eye lens cell,forming inside the lens extremely elongated fibers without nuclei and mitochondria. [8,9]

The aim of the current study is to evaluate aqueous humor activity level of SOD in primary glaucoma patients compare it with cataract group and to correlate its activity level with the duration of glaucoma

II. MATERIALS AND METHODS

Study Design

This was a case-controlled prospective study was performed in the Eye Clinic of Adam Malik Hospital comprising with primary open angle glaucoma and primary angle closure glaucoma were recruited from February until April 2017. The study was conducted in accordance with the ethical standards of Declaration of Helsinki and approved by Medical Faculty University of Sumatera Utara ethics committee. Written informed consent was obtained from all patients.

Subjects

The study was conducted on 20 primary glaucoma patients (POAG and PACG) with ages ranged 40 years to 65 years, who attended Eye Clinic of Adam Malik Hospital and who scheduled for glaucoma filtering surgey. The cataract group as a control consisted of 26 patients whose ages range from 44 to 65 years and who were scheduled for cataract surgey.

All subjects underwent ophthalmologic examination included measured of best corrected visual acuity (BCVA), intraocular pressure by non-contact tonometry (NT301-Nidek), slitlamp examination, gonioscopic (Carl Zeiss Meditec AG,

Jenna Germany), visual field with Octopus 301 and reliable SAP, optic disc with direct funduscopy (Neitz, Germany).

The inclusion criteria were primary glaucoma (POAG and PACG) patients with range age 40 to 65 years old with medically uncontrolled IOP, scheduled for surgical treatment. Patients with secondary glaucoma, previous ocular surgery, pregnant or nursing woman, patients with uncontrolled systemic diseases, and patients with history of other eye disesases or trauma were excluded from this study. Non contact tonometry was performed before and after surgery.

Aqueous Humor Sample Sampling

Aqueous humor samples were obtained from each patient requiring either elective glaucoma surgery (conventional trabeculectomy) or elective cataract surgery (alternatively phacoemulsification or extracapsular cataract extraction). Aqueous humor 0,1-0,2 ml was collected at the beginning of surgery through a paracentesis using a 27 gauge needle on a tuberculin microsyringe. Blood examination was meticulously avoided. Aqueous humor samples were immediately frozen at -80 C until processing for the subsequent biochemistry techniques. All samples were protected from light. [10]

Assay

The activity of superoxide dismutase was determined using SOD Activity assay kit Biovision K335-100 with sphectrophotometrically (Thermo Scientific) with absorbents 450 nm in aqueous humor samples.

Dilute the 1 ml of WST solution with 19 ml of Assay Buffer Solution. Centrifuge of the enzyme solution for 5 seconds. Mix well by pipetting . Dilute 15 Ul with 2,5 ml of dilution buffer. The diluted enzyme solution is stable for up to 3 weeks at 4^0 C.

SOD Assay Protocol

1. Add 20 Ul of sample solution to each sample and blank 2 well and nadd 20 Ul H2O to each blank 1 and Blank 3 well,
2. Add 200 Ul of WST Working Solution to each weel,
3. Add 20 Ul of dilution buffer to each blank 2 and blank 3 well.
4. Add 20 Ul of Enzyme working solution to each sample and blank 1 well, mix thoroughly
5. Incubate plates at 37^0 C for 20 minutes
6. Read the absorbance at 450 nm using a microplate reader
7. Calculate the SOD activity

Statistical Analysis

All data were analyzed with SPSS version 19. Data were presented as mean SD. Results ere compared by using *Mann Whitney test* to detect any differences between variables and Analysis of variance was used to detect any significant difference in both groups. P value < 0,05 was considered to be significant.

III. RESULT

Twenty eyes of 20 patients with primary glaucoma (POAG and PACG) and twenty six eyes of 26 patients with cataract as a control were included in the analysis. The clinical and demographic baseline characteristics are summarized in Table 1.

Table 1. Patients demographics and baseline characteristics

Variable	Diagnosis				*P*
	POAG	PACG	Cataract		
	n (%)	n (%)	n (%)	n (%)	
Sex					
Male	8(17.4)	2(4.3)	18(39.1)	28(60.9)	0.029*
Female	3(6.5)	7(15.2)	8(17.4)	18(39.1)	
Age					
40-60	6(13.0)	2(4.3)	6(13.0)	14(30.4)	0.137
>60	5(10.9)	7(15.2)	20(43.5)	32(69.6)	
Duration disease					
≤2 years	3(6.5)	5(10.9)	19(41.3)	27(58.7)	0.034*
>2 years	8(17.4)	4(8.7)	7(15.2)	19(41.3)	
Intraocular Preure (IOP)					
<21	1(2.2)	0(0)	26(56.5)	27(58.7)	0.0001*
22-35	4(8.7)	4(8.7)	0(0)	8(17.4)	
≥36	6(13.0)	5(10.9)	0(0)	11(23.9)	
Visual acuity					
>3/60	4(8.7)	2(4.3)	1(2.2)	7(15.2)	
3/60-1/60	0(0.0)	0(0.0)	7(15.2)	7(15.2)	0.247
<1/300	7(15.2)	7(15.2)	18(39.1)	32(69.6)	

POAG: primary open angle glaucoma
PACG; primary angle closure glaucoma
*significant at p<0,05

The table 1 based on clinical demographic, from the statistical there was significant differences from sex, duration of disease and intraocular pressure between primary glaucoma and

cataract (p<0,05), but no significant differences from age and visual acuity (p>0,05)

Table 2 SOD Activity based on sex

Sex	SOD activity (U/ml)		*P*
	n	$\bar{x} \pm SD$	
Male	28	6.971 ± 5.686	0.013*
Female	18	3.781 ± 1.608	

Mann Whitney Test

The table 2 SOD activity based on sex, there was significant differences between male and female (p<0,05)

Table 3. SOD activity based on age

Age	SOD activity (U/ml)		*P*
	N	$\bar{x} \pm SD$	
40 – 60	14	4.774 ± 3.177	0.397
> 60	32	6.137 ± 5.325	

Mann Whitney Test

The table 3 SOD activity based on sex, from the stastistical there was no significant differences from age (p>0,05)

Table 4. SOD activity in aqueous humor based on duration of disease

Duration of disease (year)	SOD Activity (U/ml)		*P*
	N	$\bar{x} \pm SD$	
≤2	27	4.995 ± 2.913	0.525
>2	19	6.756 ± 6.554	

Mann Whitney Test

The table 4 SOD activity based on duration of disease, duration disease > 2 years is more than <2 years, but from the stastistical there was no significant differences (p>0,05)

Table 5. SOD activity in aqueous humor based on intraocular pressure

Intraocular Pressure	SOD Activity(U/ml)		*P*
	N	$\bar{x} \pm SD$	
< 21	27	7.018 ± 1.119	0.076
22 – 35	8	3.779 ±1.467	
≥36	11	3.956 ± 1.583	

The table 5 SOD activity based on intraocular, IOP <21 mmHg is more compared to IOP>22 mmHg but from the stastistical there was no significant differences (p>0,05)

Table 6. Comparison SOD activity in aqueous humor between primary glaucoma and cataract

Diagnose	Aktivitas SOD		*P*
	N	$\bar{x} \pm SD$	
Primary Glaucoma	20	3.905 ± 1.459	0.02*
Senile Cataract	26	7.121 ± 5.902	

The table 6 comparison SOD activity in aqueous humor between primary glaucoma and senile cataract, SOD activity in primary glaucoma decreased compare to SOD activity in senile cataract and from the stastistical there was significant differences (p<0,05) between rhe two groups.

IV. DISCUSSION

Glaucoma continues to be a major public helath problem. It is the second leading caused of blindness worldwide after cataracts. In the United States, glaucoma is the most common eye disease and is the leading cause of irreversible blindmess in African Americans. This disease typically asymptomatic until advanced visual field occurs. Some of risk factors for glaucoma have been extensively described and studied, including elevated intraocular pressure, genetic, African American ancestry, myopia and perhaps presence of certain systemic diseases such as diabetes and hypertension.

The precise mechanism of glaucomatous damage remain unclear and is currently active focus of research. The final common pathway for all potential etiology of glaucoma is optic nerve head damage. There are many postulated mechanism of ganglion cell damage included raised IOP, genetic, vascular dysregulation, glutamate exitotoxicity, ocular ischemia, and oxidative stress are well established pathogenetic factor. [4,5,6]

Recent datas indicate that oxidative plays an important role of pathogenesis of glaucoma, but until now the mechanism is unclear. The possible caused of increased oxidative stress might be include increased of free radical or impaired antioxidant defence system. From one study reported that there was higher of lipid peroxidation concentrate in aqueous humor, trabecular meshwork and canalis schlemmi. [11,12]

In the present study, we assessed redox enzyme SOD activity in the aqueous humor of primary glaucoma and senile cataract patients.

It must be assumed that an increase in antioxidant activity can be rule within the initial steps of the oxidation process. However, a decreased antioxidant capacity of tissues and body fluids may be the consequence of long-lasting oxidative changes, as in our glaucoma/cataracts patients. [13]

Aqueous humor is known to contain several active oxidative agents, such as hydrogen peroxide and superoxide anion. It has been suggested that chronic oxidative stress insult induced by this agent can compromise trabecular meshwork ™ function. The

TM which forms the major route for the aqueous outflow from anterior chamber, contains SOD and catalase. TM is exposed to chronic oxidative stress over the course of a lifetime, and therefore, it has a sophisticated defense mechanism against reactive oxygen species. [12]

In the present study from the demographic data we found significant differences from sex, duration of disease and intraocular pressure between primary glaucoma and cataract ($p<0,05$) and there was significant differences SOD activity level based on age ($p<0,05$), whereas in male SOD activity level less than female.

In our study we found significant differences SOD activity level between primary glaucoma compare to cataract patients as a control. However, the aqueous humor activity level of SOD were significantly lower in primary glaucoma. Some authors have described the presence of oxidative agents in the aqueous humor, such as super-oxide anion and hydrogen peroxide. It has also been proposed that oxidative stress can damage the cells of the trabecular meshwork. Moreover, SOD has been localized in these cells. In another design study, Ferreira et al, observed an increase in SOD and gluthathione peroxidase activity in glaucoma compared with those with cataracts, and no significant changes were found in CAT levels. They concluded that oxidative stress might lead to an induction of antioxidant enzymes and contribute to reactive antioxidant potential decrease.[14] Superoxide dismutase may be useful as oxidative stress markers in aqueous humor of the glaucoma patients.

In conclusion suggest that lower redox enzyme is an important risk factor in the development of primary glaucoma. Assays of redox enzymes activities could provide marker to identify individuals predisposed to primary glaucoma, so further long term studies on larger population are needed for the therapy and prevention of glaucoma.

ACKNOWLEDGMENT

The authors are deeply indebted to Medical Faculty Sumatera Utara University for providing equipment and scientific apparatus

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

REFERENCES

[1] TJ Deutsch TA. Grand MG. Introduction to Glaucoma. In:Lisegang. TJ, Ed Glaucoma Basic and Clinical Science Course. San Fransisco. 2015:10:11-4

[2] Schacknow P,Samples J. The Glaucoma Book: A Practical, Evidence Based Approach to Patient Care. New York,Ny:Springer.2010:399-420

[3] Stamper R, Lieberman M, Drake M, Becker-Shaffer'S Diagnosi and Therapy of the Glaucomas. 8th Edition.New York. NY:Mosby 2009;239-265

[4] Kuehn MH, Fingerth JH, Kwon YH. Retinal Ganglion Cell Death in Glaucoma;Mechanism and Neuroprotective Strategies. Ophthalmology Clinics of North America. 2005:383-395

[5] Donne D, Rossi R,Colombo R, Giustarini D,Milzani A. Biomarker of Oxidative Damage in Human Diseases, Clical Chemistry. 2006;52:1-23

[6] Omoti AE, Edema OT.A review of the risk factors in primary open angle glaucoma. Niger J Clin Pract 2007;10:79-82

[7] Flammer J, Orgul S, Costa VP, Orzalesi N, Kreiglstein GK, Serra LM. The impact of Ocular Bloodflow in Glaucoma. ProgRetin Eye Res. 2002;21:359-93

[8] Behndig A, Svenssom B, Marklund SL, Karlsson K. Invest Ophth Vis Sci . 1998; 39:471-475

[9] Ozmen B, Ozman D, Erkin E, Guner I, Habif S, Baindir. *Clin Biochem*. 2002;35:69-82

[10] Becker-Shaffer, Aqueous Humor Formation in Diagnosis ang Therapy of the Glaucomas, 8 Edition, Mosby : Elsiver, 2009. p8-42.

[11] Kadiska MB et al. Biomarker of Oxidative Stress Study. Free Radical Bio Med. 2005; 38(6);698-710

[12] Tezzel G. Stress in Glaucomatous Neurodegenaration; Mechanism and Consequemces. ProgRetin Eye. Res.2006;25:490-513

[13] Aslan M, Cort A and Yucel I. Oxidative and nitratve stress markers in glaucoma. Free Radic Biol Med 2008;45:367-376

[14] Ferreira SM et al. Oxidative Stress Markers in Aqueous Humor in Primary Glaucoma. University of Buenos Aeros. Argentina. Elsevier. Vol 137.2004;1:62-68

AUTHORS

First Author – Masitha Dewi Sari, Department of Ophthalmolgy, Medical Faculty Sumatera Utara University Medan, Indonesia
Second Author – Hera KW Siregar, Department of Ophthalmolgy, Medical Faculty Sumatera Utara University Medan, Indonesia