EVALUATION OF OXIDATIVE STRESS MARKER AND REDOX ENZYME IN PRIMARY OPEN ANGLE GLAUCOMA

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ABSTRACT

Objective : To identify the presence of oxidative stress marker malondialdehyde (MDA) and redox enzyme gluthathion peroxidase (GPx) in primary open angle glaucoma (POAG) and relationship between oxidative stress marker malondialdehyde (MDA) and redox enzyme gluthathion peroxidase (GPx) with primary open angle glaucoma

Methods: A prospective, analytical observational with cross sectional study was conducted at the Adam Malik Hospital from January 2015 to Mei 2015 after approved by the Ethics Committee for Health Research Sumatera Utara University School of Medicine. Twenty eightpatients primary open angle glaucoma and twenty eighthealthy controls patients of match age andgenderwere included in this study prospectively.

Results: The concentration of MDA was significantly higher ($p=0,015^{*}$) and the concentration of GPx was significantly lower ($p=0,034^{*}$) in POAG group compared to control group.

Conclusions: Oxidative stress and redox enzyme is an important risk factor in the development of primary open angle glaucoma (POAG). Increased levels of oxidative stress products may be associated with primary open angle glaucoma. Therefore, assessment of oxidative stress markerand redox enzyme in POAG may be important for the therapy and prevention of glaucoma.

Keywords :malondialdehyde (MDA), gluthathion peroxidase, primary open angle glaucoma

INTRODUCTION

Glaucoma refers to a group of diseases that have common a characteristic progressive optic neuropathy with associated with visual function loss and higher of intraocular pressure as a risk factor [1].

Glaucoma usually called " the silent thief of sight" because the onset usually suddenly without symptom beforely. Glaucoma can caused irreversible blindness worldwide, including in Indonesia [2].

The pathogenesis of glaucoma is multifactorial and until now still investigate.Retinal ganglion cell (RGC) death due to apoptosis and loss of RGC axons leads to glaucomatous optic neuropathy. Many factors play role in pathogenesis glaucoma included genetic, glutamate excitotoxicity, nitric oxide and oxidative stress [3]. Oxidative stress appears play a role in progressive neuronal death that is characteristic of glaucomatous optic nerve damage [3,4]. Oxidative stress generally is induced through formation of multiple reactive oxigen species including hydrogen peroxide and superoxide that can initiate and propagate free radicals.The oxidative burden between prooxidant and antioxidant is oxidative stress that damages cellular and tissue macromolecules such as lipids, proteins and results in cellular and tissue dysfunction and cellular death [5].

Many biologic substances growth as an oxidative stress marker such asmalondialdehyde (MDA) , catalase and sodium dismutase [6]. Increased marker of oxidative stress that have been reported in glaucoma included oxidized DNA bases, lipid oxidation products and total oxidative stress marker. A number of studies in vitro and in vivo suggested that there was a role of oxidative stress marker in

glaucoma patient[5,7]. Haefiger et al reported destroyed oxidized DNA in trabecular meshwork change of matrix extracellular regulate and cytokine in glaucoma patients[7]. Flammer reported the destroyed of DNA in trabecular meshwork was higher in glaucoma patients compared to normal subjects[8].

The relationship between oxidative stress marker with glaucoma has already been reported. Flammer reported the concentration of oxidative nucleotide modification (8-OH-dG) in trabecular meshwork with glaucoma patients were correlated with the higher of intraocular pressure and decreased of visual field. Faschinger et al reported there was a positive correlation between malondialdehyde (MDA) in aquous humor glaucoma patients compared to normal subjects[7,8,9].

From these studies, the aim of the current study was to evaluate stress oxidative marker malondialdehyde (MDA)) and redox enzyme gluthathion peroxidase (GPx) in primary open angle glaucoma (POAG) and investigate the relationship between oxidative stress marker and POAG

MATERIALS AND METHODS

Subjects

This was a prospective, cross sectional study comprising twenty eightpatientspatients with primary open angle glaucoma and twenty eightcontrolsubjects as the same age range and sex were included in this study.These subjects were recruited consecutively at Haji Adam Malik Hospital North Sumatera, Indonesia. Ethical approval was obtained from University Sumatera Utara Ethics Committee. A written consent was obtained from all patients by the researchers.

All subjects underwent ophthalmologic examination included measured of best corrected visual acuity (BCVA), intraocular pressure by Goldman applanation tonometry and slitlamp examination, gonioscopic (Carl Zeiss Meditec AG, Jenna, Germany), visual field with Octopus 301 and reliable SAP, optic disc with direct funduscopy (Neitz,Japan).

Inclusion criteria: We included patients and healthy subjects who fulfilled the following criteria : age older > 40 years old, POAG patients was diagnosed based on presence of an open iridocorneal angle, the characteristic appearance of glaucomatous optic neuropathy such as enlargement of optic cup disc ratio, focal thinning of neuroretinal rim and corresponding visual field defects with Octopus 301(Haag-Streit, Interzeag International AG, Schlieren, Switzerland) and elevated intraocular pressure .

Exclusion criteria :The exception criteria included patient with cataract, ocular infection, retinopathy, taking medication as steroid and had systemic diseases such as diabetes mellitus and hypertension.

Blood Sampling

Venous blood specimens were collected from the antecubital vein into evacuated tubes 5 cc. Plasma samples obtained by centrifugation 3500 rpm. The collected venous blood were stored at 4^0C, supernatants keep in -20^0C and determination of the samples occurred within 3 months.

Laboratory Analysis

Malondialdehyde Assay

The activity of malondialdehyde (MDA) was determined by using OXIS MDA (Biotxytech). MDA was measured as thiobarbituric acid reacting substance (TBARS) production in the following manner. 0,1 ml of sample was added to a 1;1;1 (Vol/vol/vol) solution of trichloroacetic acid (15%,wt/vol),thiobarbituric acid (0,375%,wt/vol), and hydrochloric acid (0,25M). The mixture was heated at 100^0 C for 30 min. The mixture was immediately cooled and then centrifuged (3500 g for 5 min) to remove undissolved materials. Then theabsorbance at 586 nm was determined. The amount of TBARS was calculated from comparison with authenthicmalondialdehyde[10,11].

Gluthathione Peroxidase Assay

The activity of GPx was determined according to the method by Paglia and Valentine using RANSEL kit. GPx was determined spectrophotometrically by coupling the oxidation of gluthathione and NADPH

using GR. Briefly, 1 ml of assay mixture contains optimized concentrations of the following chemicals : 0,5M K2HPO4 (pH 7.0),2,5mM EDTA, 0,18U/ml GR, 100 mMgluthathione and 10 mMreduced NADPH and tissue extract 0,5mL was added in the spectrophotometer cuvette along with 0,1mL cumenehydroperoxide, a suitable substrate for GP.The mixture was placed into a 1 mL cuvette and read with Shimadzu UVPC 2100 spectrophotometer set at 340 nm at 37^0C. All chemicals were from Randox Laboratory Ltd[12].

Statistical Analysis

The collected data write in the research publication and keep in the computer. The collected data keeped in computer analysed by using the statical software. To compare quantitative variables between the two groups, unpaired t-test was used. Statistical analyses were performed with SPSS 19,0 and the level significance was P< 0,05 in all statistical test.

RESULTS

The study was conducted from January 2015 to May 2015 in 28 primary open angle glaucoma patients and 28 healthy patients

Table 1.The demographic parameters from 28 primary open angle glaucoma patientsand 28healthy control

	Primary open angle glaucoma group	Control group	P value
N	28	28	
Age	55,42 ±5,31	54,92±4,24	0,859
Sex (M/F)	14/14	15/13	
IOP right eye (mmHg)	22,64± 8,79	15,87±2,41	0,001**
IOP left eye (mmHg)	22,37± 5,52	14,21±2,53	0,001**
Systolic blood pressre (mmHg)	122,83± 5,64	123,70± 6,31	0,902
Diastolic blood pressure (mmHg)	80,25±4,91	82,6± 5,46	0,785
Serum glucose level (mg/dl)	142,21±14,45	146,71±13,27	0,321

Based on the above table appear significant difference between IOP right eye andIOP left eye in primary open angle glaucoma patients (p<0,05) compared to control groups

Table2. MDAlevels in primary open angle glaucoma group compared with control group

	MDA (nmol/L)	P value
POAG group	3,51 ± 0,79	0,015*
Control group	2,84 ± 0,58	

MDA :malondialdehyde

From the table 2, MDA significantly increased in POAG patients compared with control groups (p<0,05)

Table3.GPxlevel in primary open angle glaucoma group compared with control group

	GPx(U/gHb)	P value
POAG patients	24,52 ± 6,13	0,034*
Control patients	30.95±4,35	

GPx :Gluthathione Peroxidase

From the table 3, GPx significantly lower in POAG patients compared with control groups (p<0,05)

DISCUSSION

Glaucoma is the second leading caused blindness after cataract in the world. Glaucoma referes to a group of diseases with optic neuropathy and decreased of visual field and higher intraocular pressure as a risk factor. Glaucoma can developed after as early as birth until older age, but the most in older people.The recent study reported 61 million people had primary glaucoma and 8,4 million people result bilateral blindness[9]. In general, glaucoma classified into three major: open angle glaucoma,

closed angle glaucoma and ongenital glaucoma. Open angle glaucoma is the most type of glaucoma[10].

The pathogenesis of glaucoma is multifactorialand until now still investigate. From some literature reported glaucoma not only caused destroyed of retinal ganglion cell and its axon, but involvement optic nerve head and retina which caused blood supply decreased[13,14]. From some study also reported involvement free radical is one of pathogenesis of glaucoma, so reactive oxygen species play a role of pathogenesis of glaucoma.In ophthalmology, oxidative stress has been reported to induced the progression of cataract and diabetic retinopathy. In glaucoma, antioxidant levels decrease in aquous humor compared with normal subject. It's suggested that peroxidation involved in development of glaucoma[15].

Recent datas indicate that oxidative stress 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 defencesystem.Free radical can reacts with macromolecules as membrane lipid, protein, DNA which can changed the structure, functional and neuronal death endly[16,17]. From one study reported that there was increased of lipid peroxidation concentrate in aquous humor, trabecular meshwork and canalissclemmi primary open angle glaucoma patients compared to controls dan the study reported lipid peroxidation caused destruction of trabecular meshwork and canalissclemmi. From one study animal trial with higher intraocular pressure reported that there was an increased of MDA levels in vitreous humor[18]. MDA is a decomposition product of peroxidized polyunsaturated fatty acids.

Based on result examination, we found that MDA plasma level was increased in primary open angle glaucomagroupcompared with control group (Table 2) and GPx level was decreased in primary open angle glaucoma group compared with control group (Table 3). This result correlated with several studies have reported lower systemic levels of antioxidants in glaucoma, that is a reduced form of gluthathione level was lower in red blood cells of glaucoma patients[13]. Faschinger et al reported there was a positive correlation between malondialdehyde (MDA) in aquous humor glaucoma patients compared to normal subjects. The systemic antioxidant capacity could reflect the local ocular redox status. In experimental studies, free radical scavengers effectively prevented glaucomatous tissue such as glutamate and IOP induced RGC death, tumor necrosis factor induced axonal injury[19]Collectively decreased levels of systemic antioxidant capacity might be in the glaucomatous TM and neuronal damage as a result of local inadequate defense against oxidative stress[20,21]. Our study demonstrated that oxidative stress marker MDA and redox enzyme GPxavailable for assessing oxidative stress in POAG, but the limitation of this study the amount of this patients little, more longer times and assessing another oxidative stress marker in primary open angle glaucoma is needed.

CONCLUSION

The present study indicatesthat oxidative stress markerand redox enzyme play a role in pathogenesis of primary open angle glaucoma. Therefore, assessment of oxidative stress marker and redox enzyme in POAG may prove a valuable marker in the assessment and routine monitoring in primary open angle glaucoma. Well design, good quality prospective longitudinal trials on larger populations are therefore needed in primary open angle glaucoma and another types of glaucoma related for the therapy and prevention of glaucoma.

ACKNOWLEDGMENT

The authors are deeply indebted to Faculty of Medicine Sumatera Utara University and Adam Malik Hospital for providing equipment and scientific apparatus.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest

REFERENCES

1. TJ,Deutsch TA. Grand MG. Intraduction to Glaucoma. .In; Lisegang, TJ, ed.Gauicoma.Basic and Clinical Science Course.San Francisco. 2009;10: p 11-4

2. 2..Ministry of Health. National Strategy in Prevention Visual Impairment and Blindness to Vision 2020. 2003; p1-20.
3. 3.Dada T, Mohan S, Sihota R. Pathogenesis of Glaucoma In Sihota R, ed. Mastering the Techniques of Glaucoma Diagnosis and anagement. New Delhi: Jaypee Brothers Medical Publisher. 2008: p 128-33
4. 4.Kuehn,MH, Fingert JH, KKwon YH. Retinal Ganglion Cell Death in Glaucoma:Mechanism and Neuroprotective strategies. Ophthalmology Clinics of North America. 2005:p383-395
5. Halliwel B, GuteridgeJMC.Free Radicals in Biology and Medicine.Oxford University Press.3 rd edition. 1998; p22-24
6. .Donne D, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarker of Oxidative Damage in Human Diseases. Clinical Chemistry. 2006;52.:p 1-23
7. .Haefliger IO, Detmann E, Liu R, Meyer P, Prunte C, Messerli J, Flamer J.Nitrix Oxide and Endothelin in Pathogenesis of Glaucoma.SurvOpthalmol.1999.; p43: 51-58
8. Flammer J, Orgul S, Costa VP, Orzalesi N, Kreiglstein GK, Serra LM.The impact of Ocular Bloodflow in Glaucoma.ProgRetin eye Res..2002.: 21: p359-93
9. Muno B, West SK, Blindness and Visual Impairment in the Americans and the Carribean, British J Oph, 86.2002: p 498-504
10. Abuja MP, Albertini R. Methods for Monitoring Oxidative Stress, Lipid Peroxidation and Oxidation Resistance of Lipoproteins.ClinicaChimicaActa. 2001: p306: 1-17
11. Valenzuela A. The Biological Significance of Malondialdehyde Determination in The Assessment of Tissue Oxidative Stress. Life Science, 2001.48:p 301-309
12. Mannervick B. The Enzymes of GluthathioneMetabolism:An Overview. Biochem.Soc.Trans.1987; 15(4): p 717-718
13. Gulgun T. Oxidative stress in Glaucomatous Degeneration. Mechanism and Consequense. British J Oph, 2006: p490-513.
14. .Izzoti A, Bagnis A, Sacca SC. The Role of Oxidative Stress in Glaucoma.Mutat Res .2006; 612:105-14
15. .Boveris A. The Mitochondrial Generation of Hydroperoxide Regulation By NitrixOxide. General properties an effect of hyperbaric oxygen. Biochem J. 2000; 134: 707-16
16. .Kadiska MB et al. Biomarker of Oxidative Stress Study. Free radical Bio Med. 2005:38(6):p698-710
17. TezzelG.Oxidative Stress in Glaucomatous Neurodegeneration:Mechanism and Consequences. ProgRetin Eye Res.2006;25:490-513
18. .Ferreira SM, Lerner SF, Brunzini R, Evelson PA, Liesuy SF. Oxidative Stress Marker in Aqueous Humor of Glaucoma Patients. Am J Ophthalmol.2004;137:62-9
19. .SaccaSc, Izzoti A, Rossi P, Traverso C. Galucomatous Outflow Pathway and Oxidative Stress. Exp Eye Res.2007;84:389-99
20. Jae Hk, Louis RP. Antioxidant Intake and Primary Open Angle Glaucoma.American Journal of Epidemiology. 2003;158: 337-346
21. Gherghel D, Griffiths HR, Hilton EJ, Cunliffe IA, Hosking SL. Systemic Reduction in Gluthathione Levels Occurs in Patients With Primary Open Angle Glaucoma. Invest Opthalmol Vis Sci. 2005;46:877-83