The Potential Role of Serum Endothelin-1 Correlated with Intraocular Pressure in Primary Open Angle Glaucoma: A Cross Sectional Study in North Sumatera-Indonesia

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Abstract. Glaucoma is the second leading cause of blindness worldwide with the incidence of open-angle glaucoma more frequent than closed-angle glaucoma. Vascular endothelial cells producing peptide named ET-1, causing vasoconstrictor effects on arteries and veins with increasing blood pressure. ET-1 is considered as a potential contributor in the pathogenesis of glaucoma. This study is aimed at analysing the serum ET-1 level in Primary Open Angle Glaucoma (POAG) patients. This prospective, analytic observational with cross sectional study was conducted in Universitas Sumatera Utara Hospital and Satellite Hospital, Medan, Indonesia from December 2018 to February 2019. The sample of this study are 24 POAG patients and 24 normal subjects as a control. The patients were investigated for ophthalmology examination including visual acuity, intraocular pressure, retinal nerve fiber layer (RNFL) thickness and visual field defect. The serum ET-1 was examinated by ELISA. Serum ET-1 level correlated with higher IOP in patient with primary open angle glaucoma. Although no specific correlation can be deduced from this, effect of ET-1 still needed investigation.

Keywords: Endothelin-1, Intraocular Pressure, Primary Open Angle Glaucoma, Serum

Introduction

Glaucoma is the second leading cause of blindness worldwide with the incidence of open angle glaucoma more than closed-angle glaucoma (Kanski, 2011; Quigley & Broman, 2006). According to WHO, glaucoma causes blindness in 3.2 million people in the world. It is estimated that the number of blindness due to glaucoma in 2010 was 60,500,000, while in 2020 the number of glaucoma is estimated to increase to 76,600,000 along with the increasing population of elderly people. As much as 74% of blindness due to glaucoma originated from the primary open-angle form of glaucoma (Kanski, 2011; Quigley & Broman, 2006).

Based on RISKESDAS data in 2013, it is suspected that there are still many glaucoma sufferers who have not been detected because the symptoms that arise are often not realized so that it is diagnosed too late. In 2008, the Department of Ophthalmology, Faculty of Medicine, University of Indonesia, Jakarta Urban Eye Health Study conducted a study where the prevalence of closed- angle primary glaucoma was 1.89%, open-angle primary glaucoma was 0.48%, and secondary glaucoma was 0.16% (Kemenkes, 2015).

The pathogenesis of glaucoma is still unclear. Recent study conclude that the optic nerve damage occurs because of increased intraocular pressure, but vascular dysregulation can cause damage to the optic nerve. Apart from genetic factors, metabolic factors and toxic factors such as glutamate, NMDA, excitoxins, free radicals and nitric oxide also affect the optic nerve damage (Kuehn, Fingerth, & Kwon, 2005; Riordan-Eva & Whitcher, 2007). The potential contributor in pathogenesis of glaucoma is Endothelin-1 (ET-1). Some studies suggest that the level of ET-1 in humor aqueos is increased in patients with POAG. Endothelin is a peptide contains 21 amino acids and has two disulfide bonds in amino acids 1–15 and 3–11 with a molecular weight of 2492. The first endothelin isolated from endothelial cells is called endothelin-1 (ET-1). Furthermore, other endothelin derivatives were found called endothelin-2 (ET-2) and endothelin-3 (ET-3). ET-1 is synthesized by m-RNA of endothelial cells. ET-1 is a peptide output of vascular endothelial cells, causing vasoconstrictor effects on arteries and

vein and increasing blood pressure. When the endothelin level is too much in the blood it causes an increase in blood pressure (hypertension) and other heart diseases (Fernandez-Durango, Rollin, & Mediero, 2003; Jain, Yadava, & Raikar, 2002; Lepple-Wienheus, Becker, & Stahl, 1992; Luscher & Wenzel, 1995; Shah, 2007). Because of its potent vasoconstrictive effect, it is hypothesized that endothelin has an important role in vascular dysregulation in glaucoma patients (Shah, 2007).

ET-1 in eye is an output of non-pigmented ciliary epithelium and outpur of intraocular fluid. Its receptors are expressed on some structures, such as the muscle of ciliar body, trabecular meshwork, iris, blood vessels, retinal and optic nerve astrocytes, which determine the physiological role of ET-1 in the eye, but the role of ET-1 in the pathophysiology and physiology of IOP control remains contradictory (Jain, Yadava, & Raikar, 2002; Shah, 2007).

Kosior-Jarecka et al. (2016) reported that there was no relationship between ET-1 levels in plasma and risk factors for normal tension glaucoma. Meanwhile, according to Lie et al. (2016), from the observation normotension glaucoma patients and primary open angle glaucoma patients, the plasma ET-1 levels increased. This elevated also increases the risk of progressive normotension glaucoma & primary open angle glaucoma. As Endothelin has an important role in vascular dysregulation in glaucoma patients, as my knowledge there was no study has investigated the correlation between ET-1 and intraocular pressure in Indonesian patients, we though to analyze the potential role of serum ET-1 correlated with intraocular pressure on Primary Open Angle Glaucoma in Indonesian patients.

Methods

Study Design

This analytical prospective study used cross sectional method and consist of twenty four patients suffering from primary open angle glaucoma as a sample group and twenty four normal subjects as control group. Recruitment of these subjects were performed consecutively at Universitas Sumatera Utara Hospital in North Sumatera, Indonesia and Satellite hospital from December 2018 to February 2019. This research was established in accordance with the standards of ethics of Declaration of Helsinki and approved by Medical Faculty of University of Sumatera Utara ethical committee. Informed consent was collected from all research subjects and written in form.

Participants

The inclusion criteria in this study were Primary Open Angle Glaucoma > 18 years old without anterior segment abnormalities, cataracts, history of intraocular surgery, and history of retinal abnormalities and approved the consent form. The exclusion criteria in this study were patients with diabetes mellitus, systemic hypertension and chronic heart failure. The participants underwent routine general examination including the vitals. All subjects underwent ophthalmologic examination include visual acuity, anterior segment examination under slitlamp, IOP measurement with Goldmann Applanation Tonometer Haag Streit (R-900), Visual Field Defect with Octopus 301 Perimetry and Retinal Nerve Fiber Layer Thickness with SD Optovue OCT. The participants were investigated for plasma ET-1 examinations by the ELISA methods.

Preparation

As much as 2 mL venous blood was taken, stored in an EDTA tube, centrifuge for 15 minutes with 1000x g 2-8°C then becomes serum. It is stored at -20°C until the sample is filled and used before 7 days from the time of sampling. Before use, all serum is left at room temperature (64.4-77°F). Microplate is heated for a quarter of an hour. 30mL Wash Buffer

diluted into 750 solution with distilled water. Standard manufacturing solution with standard centrifuge 10,000 x g for 1 minute. Pour 1.0mL standard reference and sample diluents, wait 10 minutes and then turned it for several times. After that, mixed with a pipette. Produce a working solution of 80 pg / mL then make the required serial dilutions. Making gradient dilutions of 80, 40, 20, 10, 5, 2.5, 1.25, 0 pg / mL. Dilution method taken 7 EP tubes, add 500µL standard reference then dilute the sample for each tube. Suction with a 500µL pipette of 80pg / mL working solution into the tube 1 and stir it to 40pg / mL working solution. 500µL pipette from the solution from the previous tube to the next tube. Biotinylated detection Ab working solution: required sample calculation before doing the experiment (100µL / well). Centrifuge tube, diluted 100 x Biotinylated detection Ab to 1 x Biotinylated detection Ab working solution. HRP conjugate working solution concentration: calculation of samples examined before conducting the experiment (100µL / well). Prepare 100-200µL. Dissolve 100x the concentration of HRP conjugate into 1x working solution with HRP conjugate solution.

Essay Procedure

The solution concentrate is divided into 2 wells and the side (100mL per wells). Close the plate with the cover provided. Incubate the plate for 90 minutes at 37°C. Remove the liquid per wells, don't wash. Add 100µL immediately Biotinylated detection Ab working solution to each well. Close the plate with a lid, stir slowly. Incubate 1 hour with 37°C. Take the solution for each well, add 350µL of wash buffer for each well. Wash 1-2 minutes and aspirate the solution from each wells and dry. Repeat 3 times. Add 100µL of HRP to conjugate working solution to each well. Cover with a wells cover. Incubation for 30 minutes at 37°C. Aspiration of a solution for each wells. Repeat with the washing process 5 times as done in step 3. Add 90µL Substrate reagent. Cover with lid and Incubation at 37°C for 15 minutes. Do not put it in the light room. Add 50µL stop solution per wells. Using a micro-reader plate made at 450 nm, determine the optical density (OD) value of each well. Average the duplicated readings for each sample and standard. Processed in tabulated data after all results are recorded and collected.

Statistical Analysis

The data were analyzed using SPSS version 25.0 in all participant and an analytical statistic using T-test to difference in plasma level of ET-1 between the two groups and the correlation of ET-1 concentration with variables using Chi Square test and Pearson test with Confidence Interval 95% ($\alpha = 0.05$). The $p<0.05$ was considered significant.

Results

Table 1. Distribution of characteristic subjects

	POAG		Control		P
	n(%)	Mean ± SD	n(%)	Mean ± SD	
Age 18-40 years old 41-60 years old >60 years old	 3 (33.3) 14 (50.0) 7 (63.6)	52.83±9.730	 6 (66.6) 14 (50.0) 4 (36.6)	49.08 ± 10.210	0.199[a]
Gender Male Female	 12 (50.0) 12 (50.0)		 12 (50.0) 12 (50.0)		

Visual Acuity 6/6-6/18 6/18-3/60 <3/60	 7 (35.0) 12 (54.5) 5 (83.3)		 13 (65.0) 10 (45.4) 1 (16.6)		0.059
IOP ≤21 (Normal) >21 (High)	 4 (14.2) 20 (100.0)	23.46 ± 1.978	 24 (85.7) 0	14.00 ± 2.226	0.002[a]
CDR Normal: 0.2-0.3 Mild: 0.3-0.5 Moderate: 0.6-0.7 Severe: 0.8-1.0	 0 7 (35.0) 14 (100.0)	0.604 ± 0.0954	 11 (100.0) 13(65.0) 0	0.366 ± 0.0702	0.001[a]
RNFL Normal: >80nm Borderline: 70-79nm Outside normal: <60nm	 8 (25.0) 8 (100.0) 8 (100.0)	72.67 ± 12.638	 24 (75.0) 0 0	92.13 ± 6.081	0.001[a]
MD Perimetry Mild: >-6dB Borderline: -12<MD<-6dB Severe: <-12dB	 8 (25.0) 8 (100.0) 8 (100.0)	-10.01 ± 5.55	 24 (75.0) 0 0	-2.2033 ± 1.638	0.002[a]
Duration of POAG <5 years ≥5 years	 13 (100.0) 11 (100.0)				
ET-1 Level		7.163 ± 4/776		6.712 ± 3.998	0.720[b]

Note: a: Chi-square test, significant <0.05, b: T-test, significant p<0.05
Abbreviations: POAG, primary open angle glaucoma; IOP, intraocular pressure; CDR, cupping disc ratio; RNFL, retinal nerve fiber layer; MD perimetry, mean deviation perimetry; ET-1, endhotelin 1

Table 1 based on clinical demographic showed there was no significance differences from age and visual acuity, but there was significant differences from IOP, CDR, RNFL, MD perimetry between primary open angle glaucoma and controls (p<0.05). There was an increased of ET-1 concentration in POAG patients compare to controls, but from the statistical no significant differences ET-1 level between the two groups (p>0.05).

Table 2. Correlation between ET-1 Levels with CDR, IOP, RNFL, MD perimetry in POAG patients

	ET-1	
Variable	*r*	*p.value*
Visual Acuity	-0.055	0.799
CDR	0.029	0.894
IOP	0.505	0.012*
RNFL	0.000	0.999
MD Perimetry	-0.090	0.677
Duration of POAG	0.288	0.173

Note: *Pearson Chi square test, significant <0.05
Abbreviations: ET-1 level, endothelin 1; POAG, primary open angle glaucoma; IOP, intraocular pressure; CDR, cupping disc ratio; RNFL, retinal nerve fiber layer; MD perimetry, mean deviation perimetry

The current study showed a statistical significant differences between ET-1 levels and IOP ($p<0.05$) p 0.012 and have strong correlation ($r=0.054$), but no statistical significant differences between ET-1 level with Visual Acuity, CDR, RNFL, MD Perimetry and duration of POAG ($p<0.05$).

Discussion

Glaucoma is a major problem in public health. After cataracts, glaucoma is in the second place in worldwide to contribute blindness. Beside that fact, glaucoma also is the primary cause of irreversible blindness In the United States. Patients usually come to the clinic with already severe symptoms. Genetics, myopia, elevated intraocular pressure, African American race, and systemic disease become risk factors for glaucoma (Kanski, 2011; Quigley & Broman, 2006).

The research on glaucoma is still being done to determine the exact cause of the disease. Optic nerve head damage is still a major etiology of glaucoma. There are many postulated mechanisms of ganglion cell damage included vascular dysregulation, raised IOP, genetic, glutamate exitotoxicity, oxidative stress and ocular ischemia, are well rooted pathogenetic factor (Donne et al., 2006; Omoti & Edema, 2007).

Although the mechanism is unclear, oxidative plays an important role of pathogenesis of glaucoma based on recent datas. Disruption of the antioxidant defense system or an increase in free radicals are possible causes of increased oxidative stress (Kadiska et al., 2005; Tezzel, 2006).

In this study, we evaluate the concentration of ET-1 and how far the possible of concentration of ET-1 correlated with intraocular pressure in primary open angle glaucoma patients.

ET-1 is synthesized by m-RNA of endothelial cells. ET-1 is a peptide produced by vascular endothelial cells, causing vasoconstrictor effects on arteries and vein and increasing blood pressure. When the endothelin level is too much in the blood it causes an increase in blood pressure (hypertension) and other heart diseases (Fernandez-Durango, Rollin, & Mediero, 2003; Jain, Yadava, & Raikar, 2002; Lepple-Wienheus, Becker, & Stahl, 1992; Luscher & Wenzel, 1995; Shah, 2007). Because of its potent vasoconstrictive effect, it is hypothesized that endothelin has an important role in vascular dysregulation in glaucoma patients.

In this study there are significant differences from intraocular pressure, cupping disc ratio, RNFL, MD perimetry between primary open angle glaucoma and controls ($p<0.05$), but there was no significant differences ET-1 concentration between POAG and controls ($p>0.05$). In addition, ET-1 is found in the eye, which ET-1 follows intraocular fluid flow and is produce by non-pigmented ciliary epithelium. Its receptors are expressed on various structures, such as the iris, ciliary muscle, trabecular (TM) meshwork, blood vessels, retinal and optic nerve astrocytes, which determine the physiological role of ET-1 in the eye.

In our study found that there was a significant correlation between ET-1 level with IOP ($p<0.05$). This possible cause ET-1 follows intraocular fluid flow and is produce by non-pigmented ciliary epithelium and influence the intraocular pressure. From several studies that have been conducted, there are varying results. According to Lie et al. (2016), observations of patients with normotension glaucoma and primary open angle glaucoma showed increased blood levels of ET-1. According to Emre et al. (2005), patients with impaired visual field or normal intraocular pressure had high levels of ET-1 in the blood. This probably happened because the POAG patients who fulfill in this study had undergone anti-glaucoma treatment (beta blocker, acetazolamide) and filtering surgery so it is recommended that ET-1 levels be checked on newly diagnosed POAG patients (Cellini et al., 2012; Emre et al., 2005).

The limitation of our study, it wasn't a population-based study but rather hospital-based study, may cause a selection bias but futher studies are needed to prospective longitudinal clinical trials on larger population and more longer times to conclude another risk factor which correlated with pathogenesis of primary open angle glaucoma.

Conclusion

In conclusion suggest that higher ET-1 concentration is one of important developing risk factors of primary open angle glaucoma. Assays of ET-1 activities can show marker to identify individuals predisposed to primary glaucoma, further long-term studies on larger population are needed for the therapy and prevention of glaucoma.

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Conflict of Interest

The authors declare that there are no interest conflicts.

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