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REVIEW ARTICLE

THE ROLE OF LENS MALONDIALDEHYDE AND GLUTHATHIONE PEROXIDASE ACTIVITIES IN DIABETIC CATARACT AND NON DIABETIC CATARACT

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ABSTRACT

Objective: Biochemical evidence suggests that oxidative mechanisms play a major role in the etiology and pathogenesis in damage of lens proteins is involved in the genesis of senile cataract and the degenerative manifestations of diabetes such as diabetic cataract. This damage decreases the antioxidant capacity results in oxidative damage

Material and Methods: A prospective, analytical observational with cross sectional study was conducted at the Adam Malik Hospital from July 2016 to January 2017 after approved by the Ethics Committee for Health Research Sumatera Utara University School of Medicine. Fourty one diabetic cataract patients and fourty one non diabetic cataract patients of match age and gender were included in this study prospectively. Nucleus lens of malondialdehyde (MDA) and gluthathion peroxidase (GPx) who underwent cataract surgery were obtained to detect.

Results: MDA levels in diabetic cataract ranges from 1.35 to 6.4 nmol/l, with a mean deviation of 2.48 ± 0.98 nmol/l, whereas MDA levels in non-diabetic cataract ranged from 0.52 to 2.62 nmol/l with a mean of 1.45 ± 0.47 nmol/l.GPx levels in diabetic cataract ranges from 1,77 to 15,66U/L, with a mean deviation of 5,82 ± 2,40U/L, whereas GPx levels in non-diabetic cataract ranged from 7,02 to 18,41U/L with a mean deviation of 9,52 ± 2,44μm/g. These results shows a significant differences MDA level and GPx level in diabetic caratact patients compare to non diabetic cataract patients.($p<0,05$). MDA level and GPx level with the duration of Diabetes Mellitus showed significantly differences($p<0,05$)

Conclusion: Malondialdehyde and gluthathione peroxidase activities showed a decrement of antioxidant capacity in diabetic cataract that suggesting the implication of antioxidant enzymes in the genesis of diabetic cataract. Assays of malondialdehyde and gluthathione peroxidase activities could provide a marker to identify individuals predisposed to diabetic cataract

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INTRODUCTION

As age increases, cataract becomes a more common disorder. Oxidant/antioxidant imbalance plays a key pathophysiological mechanism of senile cataract genesis (Obara, 2005 and Behnding, 1998). Hydrogen peroxide is the major ocular oxidant, and can be formed in excess during the photooxidation of endogenous ascorbate and structural lens proteins, the crystallin. Exposure of the lens to hydrogen peroxide is well unknown to cause lipid peroxidation (LPO) of polyunsaturated fatty acids, loss of antioxidant, and oxidation, cross linking and insolubilization of crystallins (Virgolici, 2009). Some other oxidants like LPO adducts including hydroperoxides and malondialdehyde (MDA) also have significant cataractogenous potential and also they can be present in systemic circulation at micromocular levels and act as toxic messengers, the reports regarding the blood oxidative stress marker in patients with senile cataract are largely inconsistent (Harding, 2002; Donma, 2002; Satoh, 2004). Oxidant/antioxidant systems imbalance has also proved to have potential importance for ocular tissues such as gluthathion peroxidase (GPx), catalase, glucose-6-phosphatase dehydrogenase (G6PD) and superoxide dismutase. GPx, G6PD, catalase and superoxide dismutase have important roles in the complexly interrelated network of cellular and extracellular antioxidants. The avascular lens contains an unsually high concentration of antioxidant (Yamanaka, 2008 and Saadat, 2004). Various studies reported that persistent hyperglycemia of diabetic patients produces excess ROS leading to increased oxidative protein damage and lipid peroxidation, which would be related to the pathogenesis of diabetics complication including cataract. The toxic effects of reactive oxygen speciesare neutralized in the lens by antioxidants such as ascorbic acid, vitamin E, the gluthathione

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system (GSH peroxidase, GSH reductase) and superoxide dismutase (Simmoneli, 2009). The enzymatic (superoxide dismutase, gluthathione peroxidase, catalase) antioxidant system activities are decreased in the lens and aqueous humor during aging and in the development of senile cataract (Gort, 2001). While enzymatic antioxidant activities such as superoxide dismutase, catalase and gluthathione peroxidase decrease in liver, kidney and heart tissues of patients with diabetes mellitus, the levels of the reactive oxygen species such as malondialdehyde increase. These alterations suggest that free oxygen radicals and antioxidant mechanisms might play an important role in the pathogenesis of diabetic cataract. Futhermore, a number of studies have evaluated the oxidant markers in diabetes and its complications but with inconsistent results (Orkida Donna, 2002 and Evans, 2002). In view of these observations, the aim of this current study to evaluate the activities of oxidative marker malondialdehyde and antioxidant gluthathione peroxidase in diabetic cataract and non diabetic cataract lenses and to designated the role of these enzymes in the development of diabetic cataract.

MATERIALS AND METHODS

Subjects

This was a prospective, cross sectional study comprising fourty one patients with diabetic cataract and fourty one patients with non diabetic cataract as the same age range and sex were included in this study. These subjects were recruited consecutively at Haji Adam Malik Hospital North Sumatera and its affiliation hospital, Indonesia from July 2016 to January 2017.The diabetic patients with and without cataract attending the Endocrine and Diabetes out-patient clinic unit in Haji Adam Malik Hospital North Sumatera, Indonesia referred to the Ophthalmology Clinic to evaluate the diabetic complications such as cataract. 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

Inclusion criteria

We included patients and healthy subjects who fulfilled the following criteria: age older > 40 years old, cataract patients with best corrected visual acuity less than 6/60, visibile opacity in the lens and normal intraocular lens. For the diabetic cataract, the period of diabetes mellitus was 5 years or more. Cataract was diagnosed based on the Lens Opacitie Classification System II (LOCSII) criteria.

Exclusion criteria

The exception criteria included patient with history of steroid intake, traumatic cataract, ocular infection, and other systemic disorders. Small Incision Cataract Surgery, Extracapsular Cataract Extraction and posterior chamber intraocular lens implantation was performed on all of the cataractous patients at Haji Adam Malik Hospital North Sumatera, Indonesia. After extraction, the weighned lenses are immediately homogenized in 0,1 molar phosphate buffer PH7,0 at 0^0C. The homogenized material was stored at -20 ^{0}C until analysis.

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 trichloroaceticacid(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.

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 Mm reduced 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.

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. To analysebetween MDAlevel and MDA level with duration of Diabetes Mellitus, Anova one way 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 July 2016 to January 2017 in fourty one diabetic cataract patients and fourty one non diabetic cataract patients.

Table 1. The demographic parameters from 41 diabetic cataract patients and 41 non diabetic cataract patients

	Diabetic Cataract	Non diabetic Cataract	P
N	41	41	
Age	57,93 ±7,26	62,15 ±9,24	0,475
Sex (M/F)	23/18	20/21	
IOP right eye (mmHg)	14,64±3,79	15,87±2,41	0,862
IOP left eye (mmHg)	15,37± 2,52	14,98±2,53	0,923
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)	152,56±31,78	105,46±20,59	0,001*

Based on the above table appear significant difference between serum glucose level in diabetic cataract patients (p<0,05) compared to non cataract diabetic patients.

Table 2. MDA levels in diabetic cataract lens compared with non diabetic caratact lens

Cataract	N	MDA level		p.
		Range (nmol/L)	$\bar{x} \pm SD$	
Diabetic cataract Lens	41	1,35 – 6,40	2,48±0,98	0,0001*
Nondiabetic cataract lens	41	0,52 – 2,62	1,45±0,47	

Based on unpaired t- test, MDA levels in diabetic cataract ranges from 1.35 to 6.4 nmol/L, with a mean deviation of 2.48 ± 0.98 nmol/L, whereas MDA levels in non-diabetic cataract ranged from 0.52 to 2.62 nmol/L with a mean of 1.45 ± 0.47 nmol/L. This indicate a significant differences between MDA level in diabetic cataract compare to non diabetic cataract (p<0,05).

Table 3. GPx level in diabetic cataract lens compared to non diabetic cataract lens

Cataract	N	MDA level		p.
		Range (nmol/L)	$\bar{x} \pm SD$	
Diabetic cataract Lens	41	1,77 – 15,66	5,82±2,40	0,0001*
Nondiabetic cataract lens	41	7,02 – 18,41	9,52±2,44	

Based on unpaired t- test, GPx levels in diabetic cataract ranges from 1.77 to 15,66U/L, with a mean deviation of 5,82 ± 2,40U/L, whereas GPx levels in non-diabetic cataract ranged from 7,02 to 18,41 U/L with a mean of 9,52 ± 2,44U/L. This indicate a significant differences between GPx level in diabetic cataract compare to non diabetic cataract (p<0,05).

Table 4. MDA level with duration of diabetes mellitus

Duration of DM	N	MDA level			P
		Range (nmol/L)		$\bar{x} \pm SD$	
		Min	Max		
< 5 year	12	1,44	2,36	1,73 ± 0,25	0,0001*
5-10 year	23	1,35	3,57	2,47 ± 0,66	
>10 year	6	3,23	6,40	4,06 ± 1,17	
Total	41	1,35	2,48	2,48 ± 0,98	

Based on Anova one way test, this indicates significant differences between MDA level with duration of diabetus mellitus (p<0,05).

Table 5. GPx level with duration of diabetes mellitus

Duration of DM	N	GPx level			P
		Range (U/L)		$\bar{x} \pm SD$	
		Min	Max		
< 5 year	12	1,65	3,98	3,03 ± 2,13	0,0001*
5-10 year	23	3,74	6,27	5,54±2,45	
>10 year	6	5,95	9,46	8,06 ±2,25	
Total	41	1,77	5,82	5,82±2,40	

Based on Anova one way test, this indicates significant differences between GPx level with duration of diabetus mellitus (p<0,05).

DISCUSSION

Oxygen free radicals and antioxidant systems are thought to be involved in pathologic processes in the eye including cataract (Spector, 2005). During oxidative stress, a series of highly reactive intermediates are produced, including superoxide radical (O_2), hydrogen peroxide (H_2O_2) and hydroxy radical (OH). These radicals are capable of reacting with proteins, nucleic acids, and lipids leading to lipid peroxidation of biological membranes (Hauet, 2002). It is commonly believed that oxidative mechanisms play an important role in aetiology of cataract and also diabetic complications. The glycometabolic imbalance is also an important cataractogenic factor in diabetics. Several pathogenetic mechanisms have proposed to explair the accelerated cataractogenesis in diabetes. These mechanisms include the increased glycation and browning of lens cristallins and increased sorbitol pathway activity (Moemen, 2014). The aqueous humor normally contains hydrogen peroxide (H_2O_2), a compound capable of generating reactive oxygen species. Cataracts heve been reported to be associated with elevated level of hydrogen peroxide (H_2O_2) in aqueous humor. Crystallins and other proteins in lens fibre do not turn over and must serve the lens for the lifetime of the person. Thus the lens is even more dependent than most tissues on protection from oxidative damage.The lens defence against oxidative damage includes the enzymes GPx, SOD and CAT (Spector, 2005 and Jacques, 1998). There is evidence to suggest that GPx and SOD decreases as cataract develops including diabetic cataract. In various studies which investigated the role of oxidative stress in the development of cataract, lens lipid peroxides such as Malondialdehyde (MDA) are reported to be increased. On the other hand, an insuffuciency of enzymatic and non-enzymatic antioxidant systems with aging is also reported. Obara reported thet the lens lipid peroxides and oxidized lipoprotein levels are increased and GPx and SOD activities are decreased due to the accelerated generation of reactive oxygen species, especially hydroxyl radicals (OH) inside cataractous lenses. This author stated the lens the lens glucose,glycated protein and lipid peroxides were higher in diabetic cataract patients when compared to non diabetic cataract. In a previous study also observed higher lens lipid peride levels in diabetic cataractous lenses than senile cataractous lenses (Obara, 2005 and Ozmen, 2000).

Based on result examination, we found that lens MDA activity was higher in diabetic cataract compare to non diabetic cataract (Table 2). Katta et al reported there was a positive correlation between malondialdehyde (MDA) level in lenses diabetic cataract compared to senile cataract (p<0,05), but Agte et al reported there was no significant differences MDA level between dabetic cataract compare to non diabetic cataract (p>0,05). The lens GPx activity was decreased in diabetic cataract compare to non diabetic cataract (Table 3). This result correlated with several studies have reported lower systemic levels of antioxidants in diabetic cataract, that is a reduced form of gluthathione level was lower in red blood cells, aquoeus humor and lenses of cataract patients. PJ Hisalkar reported there was a significant differences in plasma GPx diabetic cataract patients compared to non diabetic cataract patients (p<0,005).The systemic antioxidant capacity could reflect the local ocular redox status (Hisalkar, 2012). There was a significant differences between MDA and GPx activities with duration of Diabetes Mellitus (Table 4,5). This correlated with Bron AJ et al study was reported cataract have significant correlated with age, duration of diabetes mellitus and grade of glicolited haemoglobin (Bron, 2003). Persistent hyperglycemia of diabetic patients produces excess ROS leading to increased oxidative protein damage and lipid peroxidation (Javadi,

2008). In conclusion, the increased of oxidative stress (MDA) and decrease in antioxidant (GPx) activities was more ponounced in diabetic cataract lenses than in non diabetic cataract lenses. In the light of our findings and the evidence from previous reports, we propose that diabetes itself might be a condition which leads to the acceleration of the aging process in the lens tissue. But further studies are needed to prospective longitudinal clinical trials on larger populations and longer times to conclude the exact role of oxidant stress in the development of diabetic cataract.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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