

THE CORRELATION OF HbA1c LEVEL WITH GSH-PX ENZYME ACTIVITY, AOPP, AND MDA LEVELS IN THE EYE LENSES OF DIABETIC CATARACT PATIENTS

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Abstract: *Diabetes Mellitus (DM) is known to accelerate cataractogenesis because it may cause hyperglycemia-induced stress oxidative in the eye lens. HbA1c test can be performed to monitor glycemic control. Glutathione peroxidase (GSH-Px) is an enzyme that scavenges and prevents the formation of free radical. Advanced Oxidation Protein Products (AOPP) is a marker to estimate the degree of oxidative protein modification. Malondialdehyde (MDA) can reflect cells damage. This study was an observational analytic study, conducted in September 2019-January 2020, and aimed to determine the correlation of HbA1c level with GSH-Px enzyme activity, AOPP, and MDA levels in the eye lenses of diabetic cataract patients. Data of HbA1c level and eye lenses sample was obtained from 29 cataract patients with DM who underwent phacoemulsification surgery in Ulin and Bhayangkara Hospital in Banjarmasin and fulfilled the inclusion criteria. Spearman's correlation test showed that there was no significant correlation of HbA1c with GSH-Px activity ($p=0,095$), AOPP ($p=0,084$), and MDA ($p=0,084$) levels in the eye lens of diabetic cataract patients.*

Keywords: *Diabetes mellitus, cataract, HbA1c, glutathione peroxidase, AOPP, MDA*

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia condition that refers to an increase in blood glucose levels above normal and occurs due to abnormalities in insulin secretion, its mechanism of action, or both.¹ The 2013 Basic Health Research Data stated that the prevalence of patients diagnosed with diabetes mellitus in Indonesia with age ≥ 15 years was 1.5%, whereas the prevalence in the South Kalimantan was 1.4%.² According to the data by Basic Health Research in 2018, the prevalence of diabetic patients with age ≥ 15 years in Indonesia had increased to 2% whereas in overall ages patients were 1.5%.³ Until this day, Diabetes mellitus (DM) remains a global health burden. Blood glucose control plays a crucial role to prevent complications arising from DM.¹

Monitoring and controlling diabetes mellitus can be done by measuring the blood sugar level through laboratory tests of HbA1c. HbA1c is a product from the nonenzymatic glycation process between terminal glucose NH₂ of β chain haemoglobin of valine amino acid.^{4,5} HbA1c is an excellent blood sugar control parameter because it can describe the glycemic state over the past 3 to 4 months and tends to be less fluctuative compared to other glycemic tests since it is not easily influenced by short-term lifestyle.^{4,6} Increased level of plasma glucose causes an increase in the amount of glycated haemoglobin, in which high HbA1c levels have a positive correlation with the occurrence of complications arising from diabetes mellitus.^{4,5} According to Whitting et al., an increase in HbA1c levels accompanied by a decrease in blood glucose control are related to the increased rate of antioxidant ability and the presence of oxidative stress in diabetic patients.⁷

Glutathione peroxidase (GSH-Px) is an antioxidant enzyme that catalyzes the reduction of glutathione and detoxifies

hydrogen peroxide and lipid hydroperoxide.^{8,9} This enzyme prevents the formation of new free radicals or converts free radicals into less reactive forms.^{8,10} Based on a study conducted by Giblin, glutathione peroxidase (GSH-Px), a part of the glutathione system, is highly concentrated in the lens where glutathione plays a role in oxidative stress defence which maintain normal epithelial function and lens transparency.⁹ Higher oxidative stress conditions in diabetic patients can affect the activity of glutathione peroxidase enzyme thereby accelerating the process of lens damage and cataract formation.⁸

Such oxidative stress may also involve oxidative damage against proteins. An increase in protein oxidation markers has also been linked to cataractogenesis.¹⁰ Advanced oxidation protein products (AOPP) is one of protein oxidation marker. AOPP is formed as a result of chlorinated oxidants, especially hypochlorous acid and chloramine produced by myeloperoxidase in neutrophils that are activated during oxidative stress. AOPP is defined as dityrosin which contains a cross-linked protein product and is considered as a reliable marker to measure the oxidative proteins modification level.¹¹ AOPP can cause the higher rate of reactive oxygen species (ROS) formation through the same receptor as AGEs (RAGE)¹², therefore it can also contribute to the process of cataractogenesis due to oxidative stress.

ROS is also known to be responsible for oxidative damage, with lipid peroxidation as the most significant marker, and one of them is malondialdehyde (MDA).^{13,14} Lipid peroxidation due to free radical attack of lipid membranes can produce large amounts of reactive products and is involved in the mechanism of cataractogenesis.

This study aims to determine the correlation between HbA1c levels and several indicators of oxidative stress in the eye lens, namely malondialdehyde (MDA)

levels, Advanced oxidation protein products (AOPP) levels, and the activity of the glutathione peroxidase (GSH-Px) enzyme in diabetic cataract patients.

RESEARCH METHODS

This study is an analytic observational study with a cross-sectional approach. 29 samples were obtained from the population of all diabetic cataract patients in Ulin and Bhayangkara Hospital Banjarmasin by a non-randomized sampling technique using a purposive sampling method. The inclusion criteria are in the followings: patients aged >50 years with a history of type 2 diabetes mellitus, diagnosed with cataracts and will undergo cataract surgery (phacoemulsification) by an ophthalmologist, have HbA1c data that is measured at least within one month prior to surgery and approves informed consent as a research subject. Whereas the exclusion criteria, including patients who were diagnosed and had a history of severe erythrocyte abnormalities hemoglobinopathy (thalassemia and leukemia), and severe anemia (Hb <7gr%). Data on HbA1c levels of patients in this study were taken and

collected from the medical record status of the patients. Cataract lens samples were obtained through cataract surgery by an ophthalmologist. The samples then carried out in a closed glass container containing formalin buffer to the Biochemical and Biomolecular Laboratory of the Faculty of Medicine, Lambung Mangkurat University, for measurement. The malondialdehyde (MDA) was measured by the TBARS method, AOPP levels were measured by the Cakatay¹⁵ method, and the enzymatic activity of Glutathione Peroxidase (GSH-PX) enzyme was measured by the colorimetric method. The collected data were then tabulated and analyzed using Spearman correlation test.

RESULTS AND DISCUSSION

The study regarding the relationship of HbA1c levels with GSH-Px activity, AOPP levels, and MDA lens level of diabetic cataract patients undergoing phacoemulsification has been conducted at Ulin General Hospital and Bhayangkara Hospital Banjarmasin from September 2019 to January 2020. In this study, 29 samples were obtained with the characteristics shown in table 1.

Table 1. General Characteristics of Study Subjects

Characteristic	Mean HbA1c (%)	Mean GSH-Px (unit)	Mean AOPP (mmol/L)	Mean MDA (mmol/L)	Total (n=29)	Percentage (%)
Age (yr)						
50-59	7,65	18,718	1,278	188,588	17	58,62
60-69	7,64	15,313	1,036	188,875	8	27,59
≥70	5,65	21,339	1,469	195,250	4	13,79
Sex:						
Male	7,32	19,588	1,341	192,231	13	44,83
Female	7,41	16,964	1,153	187,438	16	55,17

As the table 1 shown above, the age group in diabetic cataract patient is dominated by the 50-59 years age group in the number of 17 patients (58.62%), then followed by the 60-69 years age group as many as 8 patients (27.59%), and the lowest

number in the ≥70 years age group with 4 patients (13.79%). In this study, female patients are more commonly found (55.17%) compared to male patients (44.83%).

The highest mean HbA1c level was found in the 50-59 years age group (7.65%),

followed by the 60-69 years age group (7.64%), and the lowest in the ≥ 70 years age group (5.65%). The mean rate of glutathione peroxidase (GSH-Px) activity was highest in the ≥ 70 years age group (21,339), followed by the 50-59 years age group (18,718), and the lowest (15,313) in the 60-69 years age group. The highest mean AOPP level is in the ≥ 70 years age group (1,469), followed by the 50-59 years age group (1,278), then the

lowest in the 60-69 years age group (1,036). The MDA mean levels increase with age in the three age groups, namely the highest in the ≥ 70 years (195,250) age group, followed by the 60-69 years age group (188,875), then the lowest in the 50-59 years age group (188,588).

Table 2. The Spearman correlation test results in the correlation between HbA1c level with GSH-Px activity, AOPP levels, and MDA levels of the eye lenses of diabetic cataract patients.

Variable	Mean(+/-SD)	Median	Minimum	Maximum	<i>p</i>	<i>r</i>
HbA1c Level	7,37(+/-2,04)	6,9	5,2	14,6		
GSH-Px Activity	18,14(+/-8,08)	18,393	0,893	34,643	0,095	-0,25
AOPP Level	1,238(+/-0,44)	1,285	0,104	1,815	0,084	-0,264
MDA Level	189,586(+/-11,99)	186	178	221	0,084	-0,263

The obtained data is then analyzed using the Shapiro-Wilk test and showed that the HbA1c levels ($p=0.001$), MDA levels ($p=0.00$) and AOPP levels ($p=0.015$) were not normally distributed ($p < 0.05$), whereas glutathione peroxidase (GSH-Px) enzyme activity data were normally distributed ($p = 0.550$). According to the normality test, it was found that GSH-Px activity data are normally distributed whereas the Shapiro-Wilk test on the transformed HbA1c data remains not normally distributed ($p = 0.047$). Therefore, the three correlation tests are analyzed using the Spearman correlation test. Based on the correlation test, the results between HbA1c levels with GSH-Px activity showed $p=0.095$, with AOPP levels $p=0.084$, and with MDA levels $p=0.084$. The three results of the analysis found that there was no correlation between HbA1c levels with MDA levels, AOPP levels, and the activity of the glutathione peroxidase (GSH-Px) enzyme in the lens of diabetic cataract patients.

The results of this study are similar to the results of previous studies. Sinan Emre et al. in 2008 stated there were differences in the activity of the glutathione peroxidase (GSH-

Px) enzyme in the lens of diabetic cataracts patients compared to senile cataracts patients with $p=0.101$ ($p>0.05$).⁸ A study conducted by GN Rao et al. in 1983 found a decrease in the activity of glutathione peroxidase (GSH-Px) enzyme in the lens of cataract patients compared with the healthy senile lens.¹⁶ Whereas in the study conducted by Ilham Seghrouchni et al. in 2002, the activity of glutathione peroxidase (GSH-Px) enzyme in serum was slightly higher in diabetic patients compared with healthy subjects even though it differs insignificantly.¹⁷ The result in this study was slightly different from the study by P.H. Whitting in 2008, which stated there was a significant decrease in glutathione peroxidase (GSH-Px) activity compared to the control group with a p -value < 0.001 . However, in the said study, glutathione peroxidase (GSH-Px) enzyme activity was measured in the serum of patients.⁷

Some similar studies regarding the correlation of HbA1c levels with AOPP were conducted by Kostolanca et al. and Cakatay et al. However, in contrast to this study, which measured the AOPP levels of the lens, both of mentioned studies measured AOPP

serum levels of the patients, and HbA1c levels were divided into controlled and uncontrolled groups. The results of the study by Kostolanca et al. stated there was a significant correlation between HbA1c levels and serum AOPP levels in the group with poor glycemic control in type 1 DM patients ($r=0.447$, $p<0.01$).¹⁸ The study by Cakatay et al. found a significant correlation between HbA1c levels and serum AOPP levels in the type 2 DM group with well glycemic control ($r=0.39$, $p<0.05$).¹⁹

Based on the result of the study conducted by Bhatia et al., there was a higher increase in MDA levels in diabetic cataract patients compared with nondiabetic cataract patients. In contrast to the results of this study, the study of Bhatia et al. stated that there was a significant positive correlation between MDA and HbA1c in diabetic cataract patients with $p<0,0001$.²⁰ In line with the previous study, Altoum et al. also found a significant positive correlation MDA and HbA1c with $p=0.003$.²¹

According to the data obtained in this study, the increase in HbA1c levels in patients with diabetes mellitus cataract is not always accompanied by a decrease in GSH-Px enzyme activity and an increase in AOPP and MDA levels. This result can be caused by the fact that we did not collect the data regarding the nutritional intake of the subjects; therefore, it is unknown whether the subjects routinely consume highly contained antioxidants diets that can protect against oxidative stress. Based on the journal by Levine et al., antioxidants such as vitamin C can act as an electron donor to free radicals in the body so that it can eliminate reactive oxygen compounds in neutrophil cells, monocytes, lens proteins, and retina.¹² Popovic et al. also confirmed that the administration of vitamin C at a dose of 2 grams per day for 14 days could reduce MDA levels in plasma and suppress lipid peroxidation.²³ Another antioxidant that

might affect the results of this study is vitamin E which proved in the study by Maslachah et al. can help reduce MDA production at a dose of 1200 mg for 14 days.²⁴ However, Yasin et al., stated in his journal that the combination of 250 mg vitamin C and vitamin E 400 IU for six weeks could only inhibit the increase in the patient's plasma MDA level.²⁵ Restriction of diet consumption factors that contain antioxidants is challenging to perform; therefore, it might affect the AOPP level in the lens of the patients. Several experimental studies show that administering compounds containing antioxidants in Wistar rats such as diosmin, naringenin, resveratrol²⁶, rosmarinic acid, sinapic acid²⁷, and caffeine²⁸ can reduce AOPP levels in the lens.

The time of diagnosis, as well as the treatment, differs in each patient. Both oral antidiabetics and insulin can affect the amount of oxidative stress that occurs in the lens. In addition, different dietary intakes for each patient can also cause distinction in the amount of oxidative stress that occurs. The differences in method, sensitivity, and reagents specificity (GSH-Px kit) used in this study with some previous studies might also lead to different results. Other factors that might affect are sunlight exposure, history of smoking, and antioxidants consumption.

CONCLUSION

The average levels of HbA1c, GSH-Px enzyme activity, AOPP, and MDA in the eye lenses of diabetic cataract patients in Ulin General Hospital and Bhayangkara Hospital Banjarmasin throughout September 2019-January 2020 respectively were 7.37% ($\pm$ SD2.04), 18.14 ($\pm$ SD8,08), 1,238 mmol/L ($\pm$ SD0,44), and 189,586 ($\pm$ SD11,99). Spearman's correlation test showed that there was no significant correlation of HbA1c with GSH-Px activity ($p=0,095$), AOPP ($p=0,084$), and MDA ($p=0,084$) levels in the eye lens of diabetic cataract patients.

Suggestions for further studies regarding HbA1c and its correlation with GSH-Px, AOPP, and MDA levels can be performed with a larger number of samples and more strict criteria such as limiting the patient's antioxidant intake for a certain period of time prior to surgery. We also suggest another study regarding AOPP lens levels and their correlation with other parameters that can cause oxidative stress in cataract patients.

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