Original Article
The Relationships Between Sorbitol Dehydrogenase (SDH) Level and Diabetic Retinopathy in Diabetes Mellitus Type-2 Patients

Ramzi Amin¹, Reyno Satria Ali², Rachmat Hidayat³, Hikmat Permana³, Arief S Kartasasmita³, Dany Hilmanto⁴

¹Ophthalmology Department, Medical Faculty, Universitas Sriwijaya, Palembang, Indonesia
²Ophthalmology Department, Medical Faculty, Universitas Sriwijaya, Palembang, Indonesia
³Pharmacology Department, Medical Faculty, Universitas Sriwijaya, Palembang, Indonesia
⁴Ophthalmology Department, Medical Faculty, Universitas Padjadjaran, Bandung, Indonesia
⁵Internal Medicine Department, Medical Faculty, Universitas Padjadjaran, Bandung, Indonesia
⁶Pediatric Department, Medical Faculty, Universitas Padjadjaran, Bandung, Indonesia

ARTICLE INFO

Keywords:
Sorbitol dehydrogenase
Diabetic Retinopathy
Diabetes Mellitus

ABSTRACT

Background: The conversion of sorbitol accumulation to fructose by SDH triggers the osmotic damage in retinal endothelial cells and pericyte through the activation of advanced glycation end products (AGEs), oxidative-nitrosative stress, Protein Kinase C (PKC) pathway activation, inflammation, and the imbalance of growth factor. The osmotic damage eventually leads to DR (Diabetic Retinopathy).

Methods: This is a case-control study. DM type 2 patients who had the direct ophthalmoscopy and fundus imaging in dr. Mohammad Hoesin Central General Hospital ophthalmology outpatient in Palembang was selected as a subject in this study. Patient's blood sample was collected from a medicanubital vein for 3 mL and stored in ethylene diaminetetraacetic acid (EDTA) coated tube for SDH assay by ELISA.

Results: The average level of SDH in DR subjects was 13.3 ± 7.8 ng/mL and the average level of SDH in Non DR was 10.7 ± 2.3 ng/mL, p = 0.044. There was no significant difference in subjects with DR and subjects in control group in level of SDH.

Conclusion: There was no significant difference in subjects with DR and subjects in control group in level of SDH. The average of SDH level in DR subjects were more higher than Non DR subjects.

Introduction

Diabetic retinopathy (DR) has the potential to cause blindness in diabetes mellitus (DM) patients. The probability of this complication is escalating along with the duration of DM.¹,²,³ This complication prevalence in DM patients is 28.5% with a threat to eye vision capability for about 30%, and 15% of them are blind.²,³ The Diab Care Asia report in 2008, involving 1758 DM patients in 18 primary and secondary health service in Indonesia, revealed that 42% of DM patients will get retinopathy complication, in which a bout 6.4% is proliferative DR.⁴

Blindness caused by DR is related to the obstruction and damage in micro blood vessels in the retina. Chronic hyperglycemia condition triggers the cascade of physiology and biochemical alteration which leads to microvascular damage and retina disfunction.⁵ The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) stated that there is strong relevance between chronic hyperglycemia with DR, but the mechanism is not yet clear.⁵,⁶ Some of the biochemical pathways regarding the potential links between hyperglycemia and DR were already investigated. Under the hyperglycemic condition, glucose flux will increase via polyol pathway, where the aldose reductase (AR) enzyme will deplete the glucose supply as they are converted to sorbitol, and finally, are converted to fructose by sorbitol dehydrogenase (SDH).⁶ Quick conversion of sorbitol accumulation to fructose by SDH triggers the osmotic damage in retinal endothelial cells and pericyte through the activation of advanced glycation end products (AGEs), oxidative-nitrosative stress, Protein Kinase C (PKC) pathway activation, inflammation, and the imbalance of growth factor. The osmotic damage eventually leads to DR.⁶,⁷ The experimental study revealed some important connection between DR and SDH, which have an important role in the second part of polyol pathway.⁷,⁸ Amano et al (2007) showed that SDH was overexpressed in mammalian pericyte culture after exposed to high dose of glucose and eventually stimulated reactive oxygen. AR inhibitor and antioxidant significantly block the bad effects of excess SDH by...
preventing the loss of pericyte and vascular hyperpermeability. Loss of pericyte and vascular hyperpermeability are the initial characteristic of DR in streptozotocin-induced DM rats.

Methods

This is a case-control study. DM type 2 patients who had the direct ophthalmoscopy and fundus imaging in Dr. Mohammad Hoesin Central General Hospital ophthalmology outpatient in Palembang was selected as a subject in this study, from July 2014 – June 2016. The study had ethical clearance from Bioethics and Humaniora Unit Faculty of Medicine Sriwijaya University, Indonesia. Inclusion factor for DR group subjects was: (1) had DR based on ETDRS criteria and confirmed by direct ophthalmoscopy and fundus imaging; (2) aged 45-65 years old; (3) DM type 2 patients without any DR symptoms after direct ophthalmoscopy were considered as control. Every group has 40 subjects and their age, gender, and the DM duration were recorded.

Patient’s blood sample was collected from a median cubital vein for 3 mL and stored in ethylene diaminetetraacetic acid (EDTA) coated tube for SDH assay. Blood samples were centrifuged 10,000 rpm for 10 minutes. Supernatant was collected. Solid phase sandwich ELISA (HumanSDH ELISA kit, Abcam) were used to analysis concentration of SDH. Add samples and standards and incubate the plate at 37°C for 90 minutes, do not wash. Add biotinylated antibodies and incubate the plate at 37°C for 60 minutes, wash plate 3 times with PBS 0,01 M. Add ABC working solution and incubate the plate at 37°C for 30 minutes. Wash plate 5 times with PBS 0,01 M. Add TMB colour developing agent and incubate the plate at 37°C in dark for 20 minutes. Add TMB stop solution and read the OD absorbance at 450nm in a microplate reader. The standard curve was plotted as the OD 450 of each standard solution vs the concentration of standard solution. The human SDH concentration of the samples was interpolated from the standard curve.

The data was analyzed using SPSS 22. The relationship between SDH level and DR cases was analyzed by chi square, with 95% confidence interval. The data was shown descriptively in narration, table, and percentage.

Results:

There was no significant difference in subjects with DR and subjects in control group in gender, mean age, dan the DM duration(table 1).

Table 1. Subject’s general characteristic in DR group and control group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DR group (%)</th>
<th>Control group (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (55)</td>
<td>20 (50)</td>
<td>0,89*</td>
</tr>
<tr>
<td>Female</td>
<td>18 (45)</td>
<td>20 (50)</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>55,95±6,78</td>
<td>55,85±5,95</td>
<td>0,96**</td>
</tr>
<tr>
<td>DM duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5 years</td>
<td>29 (72,5)</td>
<td>28 (70)</td>
<td>0,76**</td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>11 (27,5)</td>
<td>12 (30)</td>
<td></td>
</tr>
</tbody>
</table>

*p<0,05, Mann Whitney Test

There was no significant difference in subjects with DR and subjects in control group in level of SDH. The average of SDH level in DR subjects were more higher than Non DR subjects (Table 2).

Table 2. SDH Level in DR and Non DR Subjects

<table>
<thead>
<tr>
<th>Level of SDH (ng/mL) ± SD</th>
<th>DR</th>
<th>Non DR</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>13,3 ± 7,8</td>
<td>10,7±2,3</td>
<td></td>
<td>0,044</td>
</tr>
</tbody>
</table>

*p<0,05, Chi square test

Discussion

Preston et al observed increased serum sorbitol in patients with type 2 DM and normal people. Mixed-meal tolerance test also showed increased sorbitol serum at meal times and during fasting. But the serum fructose level was more significantly increased in post prandial than fasting. Other study showed increased eritrocyte sorbitol at meals and fasting. Sorbitol (serum or erythrocytes) is a strong marker on polyol pathways rather than fructose, and less likely to be affected by acute metabolic status changes.

The polyol pathway is based on the enzyme aldose reductase. Aldose reductase reduces toxic aldehydes in the cell to inactive alcohols, which is its normal function. When glucose concentration in the cell becomes too high, aldose reductase also reduces that glucose to sorbitol, using nicotinamide adenine...
Microvascular endothelial cells through intracellular ROS. 

Dietary AGEs increase leukocyte adhesion to retinal diabetic complications. They are found in the retinal vessels of diabetic patients. 

AGEs increase leukocyte adhesion to retinal diabetic complications. They are found in the retinal vessels of diabetic patients. 

Hyperglycaemia inside the cell increases the synthesis of diacylglycerol, which is a critical activating cofactor for protein kinase-C (PKC). When PKC is activated by intracellular hyperglycaemia, it alters gene expression and protein function. 

Conclusion

There was significant difference in subjects with DR and subjects in control group in level of SDH. The average of SDH level in DR subjects were more higher than Non DR subjects.

References

7. Sotodeh A. The Genetic Study of Diabetic Retinopathy. Department of Ophthalmology, Faculty of Health Sciences, Flinders University, South Australia