Correlation Between Vitreous Advanced Glycation End Products, and D-dimer with Blood HbA1c Levels in Proliferative Diabetic Retinopathy

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ABSTRAK

Latar belakang: *retinopati diabetik (RD) tipe proliferatif merupakan bentuk lanjut RD yang selanjutnya dapat menyebabkan kebutaan. Kadar produk akhir glikasi/advanced glycation end products (AGEs) dan D-dimer cairan vitreus dapat menggambarkan perubahan patologi pada retina, tetapi hanya ada sedikit penelitian yang menilai korelasi antara kedua parameter tersebut dengan kadar HbA1c dalam darah. Tujuan penelitian ini menemukan hubungan antara kadar HbA1c darah dengan kadar AGEs dan D-dimer cairan vitreus pada pasien dengan RD tipe proliferatif.* ***Metode:*** *penelitian bersifat analitik potong lintang pada pasien dengan RD tipe proliferatif yang menjalani vitrektomi. Pasien dibagi dalam 2 kelompok yaitu hiperglikemi tidak terkendali (HbA1c > 7%) dan terkendali (HbA1c < 7%). Kadar AGEs dan D-dimer cairan vitreus diukur dan kadarnya dibandingkan antara pasien hiperglikemi tidak terkendali dan terkendali. Uji korelasi statistik juga dilakukan antara kadar HbA1c darah dengan kadar AGEs dan D-dimer cairan vitreus.* ***Hasil:*** *pasien berjumlah 47, dengan 32 (68.1%) pasien adalah wanita. Nilai median kadar AGEs cairan vitreus 11.0 (3.0 – 48.0) µg/mL, dan nilai median kadar D-dimer cairan vitreus 5,446.0 (44.0 – 37,394.0) ng/mL. Nilai median kadar AGEs cairan vitreus lebih tinggi bermakna pada pasien dengan hiperglikemia tidak terkendali dibandingkan dengan hiperglikemia terkendali (14.0 vs. 4.0 µg/mL; p<0.001). Terdapat korelasi positif bermakna dengan kekuatan sedang antara kadar HbA1c darah dan kadar AGEs cairan vitreus (r = 0.524; r^2 = 0.130; p=0.0001). Kadar HbA1c darah dapat digunakan untuk memperkirakan kadar AGEs cairan vitreus dengan menggunakan rumus: kadar AGEs cairan vitreus = -1.442+(1.740xHbA1c darah). Kadar D-dimer cairan vitreus pasien dengan hiperglikemia tidak terkendali tidak berbeda bermakna dengan pasien hiperglikemia terkendali (median 4607.5 vs. 5701.6 ng/mL; p = 0.458). Terdapat korelasi positif bermakna tetapi dengan kekuatan lemah antara kadar HbA1c darah dengan kadar D-dimer cairan vitreus (r = 0.342; p = 0.019). Kadar AGEs cairan vitreus memiliki korelasi positif bermakna dengan kekuatan lemah dibandingkan dengan kadar D-dimer cairan vitreus (r = 0.292; p = 0.046).* ***Kesimpulan:*** *nilai median kadar AGEs cairan vitreus lebih tinggi bermakna pada pasien RD proliferatif dengan hiperglikemia tidak terkendali dibandingkan hiperglikemia terkendali. Kadar HbA1c darah dapat digunakan untuk memperkirakan kadar AGEs cairan vitreus pada pasien RD proliferatif dengan menggunakan rumus: kadar AGEs cairan vitreus = -1.442+ (1.740x HbA1c darah). Kadar HbA1c darah pada pasien RD proliferatif tidak dapat digunakan untuk memperkirakan kadar D-dimer cairan vitreus.*

Kata kunci: *produk akhir glikasi cairan vitreus, D-dimer cairan vitreus, retinopati diabetik proliferatif, HbA1c darah.*

ABSTRACT

Background: *proliferative diabetic retinopathy (DR) is an advanced form of DR that eventually could lead to blindness. Levels of vitreous advanced glycation end products (AGEs) and D-dimer may reflect the pathological changes in the retina, but only few studies have assessed their correlation with blood hemoglobin A1C (HbA1c) levels. This study aimed to find the correlation between blood HbA1c levels with vitreous AGEs and D-dimer levels in patients with proliferative DR.* ***Methods:*** *an analytical cross-sectional study was performed in subjects with proliferative DR who underwent vitrectomy. Subjects were divided into 2 subgroups, i.e. uncontrolled (HbA1c >7%) and controlled (HbA1c <7%) groups. Vitreous AGEs and D-dimer levels were assessed; the levels were compared between uncontrolled and controlled hyperglycemic patients. Statistic correlation tests were also performed for evaluating blood HbA1c, vitreous AGEs, and D-dimer levels.* ***Results:*** *a total of 47 patients were enrolled in this study and 32 (68.1%) of them were women. Median vitreous AGEs level was 11.0 (3.0 – 48.0) µg/mL; whereas median vitreous D-dimers level was 5,446.0 (44.0 – 37,394.0) ng/mL. The median vitreous AGEs levels was significantly higher in patients with uncontrolled vs. controlled hyperglycemia (14.0 vs. 4.0 µg/mL; p<0.001). There was a significant positive correlation with moderate strength between blood HbA1c level and vitreous AGEs level (r=0.524; r^2=0.130; p=0.0001). Blood HbA1c level could be used to predict vitreous AGEs level by using the following calculation: vitreous AGEs = -1.442+ (1.740xblood HbA1c). Vitreous D-dimer levels were not significantly different between uncontrolled and controlled hyperglycemia (median 4607.5 vs. 5701.6 ng/mL; p = 0.458). There was a positive significant correlation between blood HbA1c and vitreous D-dimer levels (r = 0.342; p = 0.019); however the correlation was weak. Vitreous AGEs level had a positive significant correlation with vitreous D-dimer levels (r = 0.292; p = 0.046) and the correlation strength was also weak.* ***Conclusion:*** *median vitreous AGEs levels were significantly higher in proliferative DR patients with uncontrolled than those with controlled hyperglycemia. Blood HbA1c level can be used to assess vitreous AGEs level in patients with proliferative DR by using the following calculation: vitreous AGEs = -1.442+(1.740 x HbA1c). However, the blood HbA1c level can not be used to predict vitreous D-dimer level in patients with proliferative DR.*

Keywords: *vitreous advanced glycation end products, vitreous D-dimer, proliferative diabetic retinopathy, blood HbA1c.*

INTRODUCTION

Diabetic retinopathy (DR) is one of the major microvascular complications of diabetes mellitus, which is caused by a retinal perfusion disorder due to chronic hyperglycemia. Proliferative DR is an advanced form of DR, which is characterized by neovascularization originating from the retina and/or optic disk.[1,2] A previous study in our hospital has shown that patients with proliferative DR have increased vitreous vascular endothelial growth factor (VEGF), supporting its role in the pathogenesis of proliferative DR.[3] Various mechanisms are also involved in the pathogenesis of DR, such as activation of protein kinase C, stimulation of the polyol pathway and enhanced reactive oxygen species generation, activation of fructose-6-phosphate pathway, and increased production of advanced glycation end products (AGEs).[4,5]

In particular, high levels of AGEs may cause capillary membrane thickening and endothelial dysfunction that can stimulate coagulation system.[6,7] AGEs accumulation may also stimulate glycation process in the platelet membrane protein and cause modification of the phospholipid structure of platelet's membrane and increased platelet aggregation and adhesion leading to increased coagulation activation.[8,9] A pro-coagulant state is indeed observed in diabetes. Factors involved in coagulation system activation such as fibrinogen, factors VII, IX, etc. are usually elevated in diabetes.[10]

Elevated AGEs levels are found not only in serum but also in the vitreous.[11] The production of AGEs occurs to a much greater degree in patients with uncontrolled hyperglycemia. In diabetic patients, AGEs levels may increase to 20 folds in the vitreous humor.[12] It is suggested that vitreous AGEs influence the transition from the non-proliferative to proliferative DR.[13] AGEs

accumulation in the vitreous humor may cause chronic inflammation, neurodegeneration and retinal microvascularization dysfunction, which eventually impair vitreous permeability.[14]

D-dimer level has been used as a biomarker of hypercoagulability and fibrinolytic activity since it is a product of fibrin degradation.[15] Study assessing D-dimer from vitreous humor is scarce, but it is suggested that high level of vitreous D-dimer may indicate a substantial damage of the blood-retinal barrier because it has large molecular size (molecular weight of 180 kDa) to penetrate into the vitreous body.[16] Therefore, it is an important biomarker to indicate a more advanced stage of DR.

Currently, there is no method to predict vitreous AGEs levels or fibrinolytic activity from blood biomarkers. This study aimed to find the correlations between blood HbA1c levels, vitreous AGEs and vitreous D-dimers levels in patients with proliferative diabetic retinopathy.

METHODS

This was an analytical, cross-sectional study, which was conducted at the Department of Clinical Pathology, Cipto Mangunkusumo Hospital (CM Hospital) Jakarta, between January and June 2017. Subjects were proliferative DR patients recruited from Department of Ophthalmology, CM Hospital who were enrolled in the previous study[3] and underwent vitrectomy. Vitreous specimens were stored at -80^{o}C in the Laboratory of Clinical Pathology Department, CM Hospital.

Patients' demography, clinical data and HbA1c levels were retrieved from medical records. Patients with HbA1c levels of <7% was designated as controlled hyperglycemia, whereas HbA1c of >7% was categorized in the uncontrolled hyperglycemia group. Ethical approval was issued by the Ethical Committee, Faculty of Medicine, Universitas Indonesia by letter No. 487/UN2.F1/ETIK/2017.

Measurement of Intravitreal AGEs and D-dimer

Intravitreal AGEs levels were measured using enzyme-linked immunosorbent assay (ELISA) method with a commercial kit (OxiSelectTM Advanced Glycation End Product Competitive ELISA kit, Cell Biolabs Inc., USA). The result was expressed as µg/mL. Intravitreal D-dimer levels were measured using enzyme-linked fluorescence assay (ELFA) method with a commercial kit (VIDAS® D-Dimer Exclusion II ASSAY, Bio Mérieux, France) and the results were expressed as ng/mL. The reference value for plasma D-dimer was <300 ng/mL.

Statistical Analyses

Patients' demography and clinical data were presented descriptively. Vitreous AGEs and D-dimer levels were expressed as median and range due to their skewed distribution. Median differences between groups were analyzed using Mann-Whitney U test. A p value of less than 0.05 was considered significant. Correlation tests were performed using Spearman's rho correlation coefficient for skewed data. Statistical analyses were done using a statistical software SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

A total of 47 patients were enrolled in this study and 32 (68.1%) of them were women. Mean age of the patients was 53.0 (SD 8.03) years old. Most patients had uncontrolled hyperglycemia (HbA1c > 7%) and had been diagnosed for less than 15 years (**Table 1**).

Table 1. Subject characteristics (n=47)

Variables	Median (range)	n (%)
Gender		
- Male		15 (31.9)
- Female		32 (68.1)
Glycemic control (HbA1c)	8.4 (5.4–13.5)	
- Controlled (HbA1c <7%)		11 (23.4)
- Uncontrolled (HbA1c >7%)		36 (76.6)
Duration of diabetes	10 (1–35)	
- <15 years		33 (70.2)
- ≥15 years		14 (29.8)

Vitreous AGEs and Vitreous D-dimer Levels

Median vitreous AGEs level was 11.0 (3.0 – 48.0) µg/ mL, whereas the median vitreous D-dimer level was 5,446.0 (44.0 – 37,394.0) ng/ mL. Thirty-four (72.3%) patients had D-dimer level of >300 ng/mL.

Correlation Between Blood HbA1c and Vitreous AGEs and Vitreous D-dimer Levels

Vitreous AGEs levels were significantly higher in patients with uncontrolled (HbA1c >7%) than controlled hyperglycemia (HbA1c <7%). The median was 14.0 vs. 4.0 μg/mL (p<0.001) for uncontrolled and controlled hyperglycemia, respectively (**Figure 1**). There was a significant positive correlation with moderate strength between blood HbA1c level and vitreous AGEs level (r=0.524; r^2=0.130; p=0.0001). Blood HbA1c level could be used to predict vitreous AGEs level by the following calculation:

Vitreous AGEs = -1.442 + (1.740 x HbA1c)

Vitreous D-dimer levels were not significanly different between patients with uncontrolled and controlled hyperglycemia (median 4607.5 vs. 5701.6 ng/mL; p=0.458) (**Figure 2**). There was a significant positive correlation with weak strength between HbA1c and vitreous D-dimer levels (r=0.342; p=0.019).

Vitreous AGEs was weakly correlated with vitreous D-dimer levels (r=0.292; p=0.046). Patients with vitreous D-dimer of more than 300 ng/mL tended to have higher vitreous AGEs level than those with D-dimer of ≤300 ng/mL (12 vs. 10 μg/mL; p=0.073).

DISCUSSION

This study is the first to evaluate the correlation among intravitreal AGEs, D-Dimer

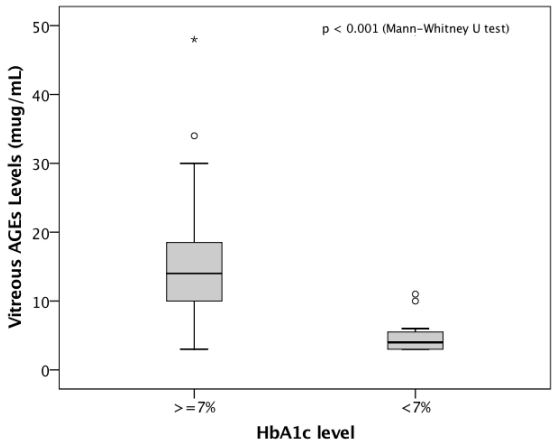

Figure 1. Vitreous AGEs levels between patients with uncontrolled (HbA1c ≥7%) and controlled (HbA1c <7%) hyperglycemia

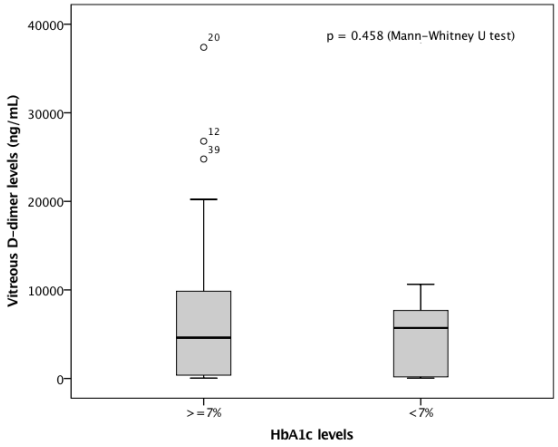

Figure 2. Vitreous D-dimer levels between patients with uncontrolled (HbA1c ≥7%) and controlled (HbA1c <7%) hyperglycemia

levels and blood HbA1c in Indonesian patients with proliferative DR. To our knowledge, this study also provided first data on vitreous AGEs and D-dimer levels comparing controlled and uncontrolled hyperglycemic DR patients. Female patients were predominant in this study, which is similar to another study about prevalence and risk factors for DR in Malay ethnic, which was conducted in Singapore (60-70%).[21] The mean age of patients was also comparable with other Asian populations with DR.[22]

Our study has shown that high HbA1c was moderately associated with higher vitreous AGEs levels. This supports the theory that uncontrolled hyperglycemia has an important role in the pathogenesis of proliferative DR.[13] Moreover, it can be said that we are able to predict vitreous AGEs levels based on HbA1c levels obtained from a blood sample taken from the patients. This finding implies that HbA1c levels can be used to monitor disease progression in an individual patient.

High vitreous D-dimer levels in this study suggest that there was increased fibrinolytic activity in proliferative DR patients. A previous study has reported increased components of the fibrinolytic system, including D-dimer, in the vitreous body of vitreoretinal disorders. The mean vitreous D-dimer level was 1.64 μg/mL (1,640 ng/mL) in patients with proliferative vitreoretinal disorder.[16] The high vitreous D-dimer levels might be surprising since in another study, blood D-dimer levels in diabetic patients with DR was

similar with diabetic patients without DR (0.2 vs. 0.2 μg/mL; p = 0.960). The breakdown of the blood-retinal barrier is thought to be responsible for increased vitreous D-dimer levels and also other components of the fibrinolytic system.[16]

The wide range and skewed distribution of vitreous D-dimer levels may affect the statistical correlation test. The regression test was performed based on an assumption that numerical data have linear distribution, but we observed that high vitreous D-dimer levels could be found in patients with normal HbA1c levels. Other factors are involved in the pathogenesis of coagulation and fibrinolytic activities and these may influence the D-dimer levels in the vitreous humor.

This study has a limitation that it was not designed prospectively to provide evidences that uncontrolled hyperglycemia causes increased vitreous AGEs levels or fibrinolytic activity. Prospective studies may not be suitable due to ethical problem to obtain vitreous specimens from asymptomatic DR subjects. However, this study may suggest that it is important to control hyperglycemia to prevent the increase of vitreous AGEs level. On the other hand, abnormalities of coagulation and fibrinolytic activity can still be present even in patients with good glycemic control. This is supported by a study showing that coagulation and fibrinolytic abnormalities has a strong association with the presence of diabetic vascular complications and the association was stronger than the degree of glycemia.[24]

CONCLUSION

Median vitreous AGEs levels are significantly higher in patients with uncontrolled than those with controlled hyperglycemia. Blood HbA1c levels can be used to assess vitreous AGEs level by the following calculation: vitreous AGEs = -1.442+(1.740 x HbA1c). Blood HbA1c level can not be used to predict vitreous D-dimer level.

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