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# The risk of elevated plasma fibrinogen level in hypertensive and normotensive patientsafter bevacizumabintravitreal injection in diabetic retinopathy

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## ABSTRACT

Bevacizumab intravitreal injection (IVB) could be detected in plasma that might cause an imbalance in the coagulation system. A hypercoagulable state is potentially involved in the risk for thrombosis, which is associated with high cardiovascular mortality.The objective of the current study was to investigate the risk of elevated plasma fibrinogen levelsin hypertensive and normotensive patients after IVB in diabetic retinopathy. This study was conducted at Dr. Sardjito General Hospital, Yogyakarta from March to June 2019. A total of 64 patients were enrolled in the study, included of 32 hypertensive and 32 non-hypertensive patients with diabetic retinopathy who underwent IVB. Patients were interviewed and investigated for physical condition and opthalmological examination. Fibrinogen levelwas measured before and 1 week after IVB. The mean fibrinogen level beforeand after IVB was slightly high in hypertensive patients than normotensive but not significantly different($p>0.05$). There was no significant risk of increased fibrinogen levels after IVB in the hypertension group compared to the normotension group in diabetic retinopathy patients. The proportion of patients at high risk for cardiovascular disease after IVB was not significantly different between both groups.

## ABSTRAK

Injeksi intravitreal bevacizumab (IVB) dapat dideteksi dalam plasma, yang dapat menyebabkan ketidak seimbangan dalam sistem koagulasi. Hiperkoagulasi berpotensi menimbulkan trombosis, yang dikaitkan dengan tingginya mortalitas kardiovaskular. Tujuan penelitian ini adalah mengkaji risiko peningkatan kadar fibrinogen plasma pasien hipertensi dibandingkan normotensi setelah pemberian IVB pada retinopati diabetika. Penelitian dilakukan di RSUP Dr. Sardjito, Yogyakarta bulan Maret hingga Juni 2019. Total sebanyak 64 pasien, terbagi menjadi kelompok hipertensi (n=32) dan normotensi (n=32) terlibat dalam penelitian. Pasien diwawancara dengan menggunakan kuesioner kemudian dilakukan pemeriksaan fisik dan status oftalmologis. Kadar fibrinogen diperiksa sebelum dan 1 minggu setelah IVB. Rerata kadar fibrinogen sebelum dan setelah IVB lebih tinggi pada pasien hipertensi dibandingkan pasien normotensi tapi tidak berbeda bermakna ($p>0.05$). Tidak didapatkan risiko peningkatan kadar fibrinogen yang signifikan setelah IVB pada kelompok hipertensi dibandingkan normotensi pada pasien retinopati diabetika. Proporsi pasien yang berisiko tinggi menderita penyakit kardiovaskular setelah injeksi IVB tidak berbeda bermakna antara kedua kelompok.

***Keywords***:
fibrinogen;
hipertensi;
bevacizumab;
IVB;
retinopati diabetika

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## INTRODUCTION

Intravitreal injection has been known since 1911 for retinal detachment therapy with injecting air to the vitreal cavity. Since then, intravitreal injection has been usedto treat various intraocular abnormalities.[1,2] Frequency of intravitreal injection utilization increases with the introduction of anti-vascular endothelial growth factor(anti-VEGF) therapy. Anti VEGF therapy is used widely for the treatment ofchoroidal neovascularization (CNV) secondary to pathological myopia, idiopathic CNV, diabetic retinopathy with macular edema, retinal vein occlusion, and any other chorioretinal vascular abnormalities.[2,3]

Bevacizumab (Avastin®) works by inhibiting the process of angiogenesis, a physiological process of new blood vessels formation targeting VEGF-A.[4] Bevacizumab also has a role in stimulating regression of microvascular abnormalities, prevent bleeding and inflammation, also stabilize the normal vascular.[5]

The administration of bevacizumab can cause an imbalance in the coagulation system.Systemic inhibition of VEGF-A is associated with an increased risk of gastrointestinal bleeding, the incidence of arterial thrombosis, and death. The appearance of the blood-retinal barrier restricts the release of anti-VEGF agent to the blood flow so it can minimize systemic absorption. Intravitreal dose of bevacizumab is 400 times smaller than intravenous dose. So, intravitrealinjection is a safer method in anti VEGF therapy.[2] Several studies have shown that intravitreal anti-VEGF agents may be detected in a patient's plasma because in neovascular abnormalities, blood-retinal barrier damage can occur thus allowing the absorption of anti-VEGF into the systemic circulation.[6] Based on this, systemic safety requires great attention in patients who received anti-VEGF therapy.[2]

Hypertension is a chronic medical condition of the heart and an increase in arterial blood pressure.Hypertension is the most important risk factor in cardiovascular disease-related blood coagulation system disorders. Damage of the tunica intima in hypertension patients causes atherosclerosis, which can increase platelet aggregation that triggers heart and blood vessel diseases.[7]

Clotting time, plasma fibrinogen levels, and blood viscosity are the most common screening tests to determine of pre-thrombosis status.[2] The effect of increasing fibrinogen levels is increasing blood viscosity, fibrin clot size, tissue deposition, stimulation of atherosclerosis, and vascular thickening which involved in the pathogenesis of cardiovascular thrombosis events.[8] The study aimed to investigate the risk of elevated plasma fibrinogen levelsin hypertensive and normotensive patients after bevacizumab intravitreal injection (IVB) in diabetic retinopathy.

## MATERIALS AND METHODS

### Subjects

This study was conducted atDr. Sardjito General Hospital, Yogyakarta, Indonesia from Marchto June 2019. Subjects were interviewed using questionnaires, underwent physical and ophthalmological examinations, then examined for fibrinogen level beforeand one week (6-9 days) after IVB. The subjects who met the inclusion and exclusion criteria were enrolled in the study. The inclusion criteria were patients aged 40-65 years old, can be interviewed, are willing to do laboratory tests and controlling examination on schedule. The exclusion criteria included anticoagulant therapy (heparin or warfarin), history of coagulation disorders, and patients whose blood samples could not be carried out by a laboratory test. The subjects were then

grouped into two groups included of 32 hypertensive and 32 normotensive patients with diabetic retinopathy who underwentIVB. The hypertension group consisted of diabetic patients with blood pressure at examination ≥140/90 mmHg and have history of hypertension. The normotension group consisted of diabetic patients with blood pressure <140/90 mmHg and have never been diagnosed with hypertension by a medical doctor.

**Fibrinogen analysis**

Blood samples were drawn from the mediancubital vein using sterile disposable puncture needles, and placed in 2mL tubescontaining trisodium citrate 3.2% (0.109M) which have a ratio of 9:1. The sample were centrifuged for 15 min at 3500 rpm. Citrate blood was prepared into plasma citrate then fibrinogen was immediately examined using a full automatic coagulometer ACLTop 300 (IL).

**Statistical analysis**

Data were presented as mean ± standard deviation (SD) or frequency or percentage. The different of the plasma fibrinogen levels and other variables of the hypertensive and normotensive groups was analysis using student t test. A p value < 0.05 was considered as significant.

## RESULTS

Patients demographic and clinical characteristics are summarizedinTABLE 1. There was no significant differences in baseline values between groups (p> 0.05) except for smoking history (p = 0.039).

TABLE1. Demographic and clinical characteristic subjects

| Characteristics | Hypertension | Normotension | p |
|---|---|---|---|
| Age(years) | 55.78±5.14 | 53.53±6.94 | 0.146 |
| Gender | | | |
| • Male | 13(40.6) | 20(62.5) | 0.080 |
| • Female | 19(59.4) | 12(37.5) | |
| Visus (logMar) | 0.49±1.64 | 1.04±0.87 | 0.098 |
| Duration of DM (years) | 9.50±6.49 | 9.87±7.32 | 0.946 |
| Regular antidiabetics | | | |
| • Yes | 30(93.8) | 31(96.9) | |
| • No | 2(6.2) | 1(3.1) | 1.000 |
| Smoking history | 4(12.5) | 0(0.0) | 0.039 |
| Dyslipidemia | 13(40.6) | 11(36.7) | 0.749 |
| BMI | 24.39±3.07 | 23.36±3.58 | 0.221 |
| PE History | | | |
| • Everyday | 10(31.3) | 4(12.5) | |
| • 1-3time/week | 6(18.8) | 6(18.8) | |
| • Infrequently | 16(50.0) | 22(68.8) | 0.172 |
| Fibrinogen level before IVB (mg/dL) | 364.22±47.48 | 352.59±43.57 | 0.311 |
| Total of IVB | 3.00±2.87 | 4.031±4.81 | 0.743 |
| IOP before IVB (mmHg) | 13.17±2.78 | 13.00±3.41 | 0.802 |

DM = Diabetes Melitus; BMI = Body Mass Index; IVB = Intravitreal Bevacizumab; PE = Physical Exercise; IOP= Intraocular Pressure. Data on age, vision, duration of DM, BMI, pre IVB fibrinogen level, IVB count and IOP pre IVB are displayed as mean ± standard deviation (SD). While data on sex, regular antidiabetics, smoking history, dyslipidemia and PE history werepresented in frequency and percentage (n,%)

TABLE 2 shows that fibrinogen levels before and after IVB. In the hypertension group had the fibrinogen levels higher than that the normotension group. However,it was notsignificantly different (p> 0.05).

Patients with elevated fibrinogen levels were slightly higher in the hypertension group than normotension group (TABLE 3), with an RR value of 1.182 and 95% CI (0.626-2.233). However, there was no risk relationship associated with an increase in fibrinogen levels in the both groups.

TABLE 2. Fibrinogen levelbefore and after IVB in hypertension and normotension groups

| Group | Fibrinogen levels (mean±SD) | | p |
|---|---|---|---|
| | Before IVB | After IVB | |
| Hypertension(n=32) | 364.22±47.48 | 363.06±53.32 | 0.882 |
| Normotension(n=32) | 352.59±43.57 | 346.18±51.27 | 0.237 |
| p | 0.311 | 0.103 | |

SD = standard deviation

TABLE 3. Proportion of patients who had elevatedfibrinogen level afterIVB

| Group | Fibrinogen levels [n (%)] | | RR (95 %CI) |
|---|---|---|---|
| | Elevated | Unelevated | |
| Hypertension (n=32) | 13 (40.6) | 19 (59.4) | 1.182 (0.626-2.233) |
| Normotension(n=32) | 11 (34.4) | 21 (65.6) | |

RR = Relative Risk; CI=Confidence Interval

TABLE 4. Relative Risks (RR) of subjects related to cardiovascular disease after IVB

| Group | Risk of cardiovascular disease [n (%)] | | RR (95 %CI) |
|---|---|---|---|
| | High | Low | |
| Hypertension (n=21) | 19 (90.5) | 2 (9.5) | 1.131 (0.847-1.509) |
| Normotension(n=15) | 12 (80.0) | 3 (20.0) | |

RR = Relative Risk; CI=Confidence Interval; High Risk= fibrinogen levels≥350 mg/dL, Low Risk= fibrinogenlevels≤290 mg/dL)

The subjects consisted of both groups having high risk (fibrinogen levels ≥350 mg/dL) and low risk (fibrinogen levels ≤290 mg/dL) of cardiovascular disease. TABLE 4 shows, hypertension condition did not increase the risk of cardiovascular disease. TABLE 5 compares the risk of increasing fibrinogen levels in patients who have received first IVB and more than three times. There was no significantly different found in increased level of fibrinogen in subjects from the both groups. This showed, repeated or chronic IVB administration did not raise the risk of increased fibrinogen level with RR value of 1.064 and 95%CI (0.529-2.140).

TABLE 5. Risk of elevated fibrinogen level based on frequency of IVB administration

| Variable | Fibrinogen level [n (%)] | | RR (95 %CI) | p |
|---|---|---|---|---|
| | Elevated | Unelevated | | |
| IVB > 3 time (n=22) | 9 (40.9) | 13 (59.1) | 1.064 (0.529-2.140) | 0.863 |
| First IVB(n=26) | 10 (38.5) | 16 (61.5) | | |

RR = Relative Risk; CI=Confidence Interval

## DISCUSSION

There were four study subjects with smoking history in the hypertension group. Several studies showed a close relationship between increased blood pressure and history of smoking. Older men with history of moderate to severe smoking have significantly higher systolic blood pressure than non-smokers.[9]

Fibrinogen levels in the hypertension group, were slightly higher than the normotension group as shown in TABLE 2. Proportion test result showed that there was no risk relationship between increased level of fibrinogen in the two groups. Hypertension is not only one of the most important risk factor for cardiovascular disease, but also the most modifiable risk factor for stroke. There is a change in the blood clotting system in hypertensive patients.[7] Fibrinogen was identified as the major independent risk factor for cardiovascular disease.[10] Eldour *et al*,[7] found that plasma fibrinogen level significantly higher in hypertensive patients than in the control group. Secchi *et al*,[11] reported a strong and independent relationship between fibrinogen and the severity of damage correlated with hypertension in different target organs.

Majeed *et al*,[8] mentioned that fibrinogen level can affect the prognosis of hypertensive patients. Hypertensive patients with plasma fibrinogen more than 350 mg/dL have a risk of cardiovascular disease 12 times greater than hypertensive patients who have fibrinogen under 290 mg/dL.[12] TABLE 4 showed that the hypertension condition does not increase the high risk of cardiovascular disease. Majeed *et al*,[8] also showed that there is no significant difference in fibrinogen levels between the hypertension group and the normotensive population. This result can be caused by differences in patient's blood pressure, smaller sample size, and the presence of antihypertensive drugs in the hemostatic system.

Bevacizumab vitreous half-life has been estimated at 11.3 days after intravitreal injection.[13,14] The maximum serum concentration (3.3 µg/mL) was reached in 8 days after IVB, with concentration that remained above 1µg/mL after 29 days. Bevacizumab elimination from aqueous humor and serum was the same as vitreous which have a half-life of 4.88 days and 6.86 days.[15,16] The systemic concentration of bevacizumab after 1.25 mg intravitreal injection ranged from 59.8 to 86.5 ng/mL and its ability to bind with the VEGF was significantly lower than ranibizumab due to the maturation of bevacizumab affinity was 14-100 times lower.[17,18]

The causes of fibrinogen levels after IVB were not significantly different between the two study groups might be caused by very low doses, intravitreal routes of administration (there is a blood-retinal barrier) and low bevacizumab affinity. Insignificant fibrinogen levels between the two groups might also be caused by condition of diabetes mellitus of the subjects. All subjects in this study were diabetes mellitus sufferers who had both hypertension and normotension. Increased level of fibrinogen or

hyperfibrinogenemia, can occur in people with diabetes mellitus whether they are related to hypertension or not, depending on the condition of diabetes itself.

The various possible mechanisms for hyperfibrinogenemia in diabetics could be that a procoagulant state often exists in people of diabetes. There is an increase in some coagulation factors such as plasminogen activator inhibitor 1, von-Willebrand factor, fibrinogen, factor VII and thrombin-antithrombin complexes particularly in association with macrovascular and microvascular disease and glycemiccontrol. Plasma levels of lipoprotein(a) [Lp(a)] are elevated in people with diabetes, particularly those with poor glycemic control. The Lp(a) molecule is formed by the assembly of at least two major proteins, a molecule of apoB100 covalently linked to a molecule of apolipoprotein(a) [APO(a)] by a single disulfide bridge. It is structurally similar to low-density lipoprotein (LDL) in protein and lipid composition, the essential difference between the two being APO(a). APO(a), a glycoprotein structurally similar to plasminogen, the precursor of plasmin can bind to fibrin, the membrane protein of endothelial cells and monocytes. This inhibit plasminogen binding and plasmin generation which leads to decreased fibrinolysis and delayed thrombolysis and contributes to the accumulation of Lp(a) and fibrin at the sites of vascular injury. Lp(a) has a major role in diabetes and its vascular complications by decreasing fibrinolysis and thus increasing plasma fibrinogen levels.[19]

The correlation between glycemic control and fibrinogen levels could be due to two reasons. Either glycosylate fibrinogen is less susceptible to plasmin degradation or relative insulin deficiency in diabetic's results in differential protein synthesis i.e., 29% decrease in albumin synthesis and 50% increase in fibrinogen synthesis.[19,20]

The limitation of this study was that there was no further investigation of the diabetes mellitus conditionsuch as duration of illness, adherence to taking medication and HbA1c levels), severity of hypertension and adherence in taking antihypertensive drugs.

## CONCLUSION

There is no significant risk of increased fibrinogen levels after IVB in the hypertension group compared to the normotension group in diabetic retinopathy patients. Further research are suggested to investigate the relatioship of diabetes mellitus conditions such as duration of illness, compliance with taking medication and HbA1C levels, severity of hypertension and adherence in taking antihypertensive drugs.

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