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Shortening Activated Partial Thromboplastin Time (APTT) Between Hypertensive and Non-hypertensive in Diabetes Mellitus Patients with Bevacizumab Intravitreal Injection (IVB)

Anik Ika Winarni[1], Ni Luh Putu Widyasti[1], Natalia Christina Angsana[2], Rizto Wisuda Senuari[2], Angela Nurini Agni[1], Agus Supartoto[1], Muhammad Bayu Sasongko[1], Haryo Yudono[1], Retno Ekantini[1], Usi Sukorini[3]

[1]*Department of Ophthalmology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada-Dr. Sardjito General Hospital, Yogyakarta, Indonesia*
[2]*Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[3]*Department of Clinical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada-Dr. Sardjito General Hospital, Yogyakarta, Indonesia*

ABSTRACT

***Introduction**: Bevacizumab intravitreal injection could be detectable in plasma that might interfere with the coagulopathy and hemostasis condition. The purpose of this study was to investigate the difference of APTT between hypertensive and non-hypertensive diabetes mellitus patients with bevacizumab intravitreal injection.*

***Methods**: This cohort study was conducted at Sardjito General Hospital from March 2019 to June 2019. Thirty-two hypertension patients and 30 non-hypertension patients with diabetes mellitus who underwent bevacizumab intravitreal injection were included. The value of APTT was measured using ACLTOP300 machine before and one week after IVB. The difference in mean APTT value before and after IVB, range APTT value between two groups were assessed using independent t-test. The percentage of patients who had shortening of APTT in both groups was tested by two population proportion test.*

***Result**:* Mean APTT before IVB in hypertensive patients was 36.47 ± 2.92 seconds and in non-hypertensive patients was 36.33 ± 4.39 seconds with p value > 0.05. The mean value of APTT after IVB in hypertensive patients was 35.42 ± 3.63 seconds and in non-hypertensive patients was 35.60 ± 3.13 seconds with p value > 0.05. APTT shortening in hypertensive patients was -1.03 ± 3.65 and non-hypertensive patients was -0.73 ± 2.55 with p value > 0.05. The risk of APTT shortening in hypertensive patients was 1.370 (0.831-2.258). The risk of APTT shortening in hypertensive patients who used antihypertensive drugs regularly was 0.538 (0.331-0.874).

***Conclusion**:* There was no difference in the shortening of APTT value one week after intravitreal bevacizumab injection between hypertensive and non-hypertensive groups in patients with diabetes mellitus. Hence, the administration of IVB in hypertensive patients with regular antihypertensive therapy might be safe.

Keywords: *APTT, hypertension, bevacizumab, IVB, diabetes mellitus*

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INTRODUCTION

Intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) agents have revolutionized the treatment of retinal diseases, including neovascular age-related macular degeneration (AMD), diabetic retinopathy, retinal vein occlusions, Retinopathy of Prematurity (ROP), moreover promising results were reported in neovascular glaucoma and intraocular tumor[1]. In 2005, Rosenfeld et al reported that intravitreal injection of bevacizumab could improve vision and reduce macular swelling in AMD, then off-label of bevacizumab intravitreal became a therapeutic choice[2].

*Correspondence to:
Anik Ika Winarni,
Department of Ophthalmology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada,
anik.ika.w@gmail.com

Bevacizumab is an anti-VEGF agent which function not only prevents blood vessel growth and neovascularization but also triggers the regression of microvascular abnormalities, stabilizes normal blood vessels, and prevents bleeding and inflammation. The molecular weight of bevacizumab is 148 kDa (two times greater than ranibizumab) which is in ophthalmology is still widely used because it is cheaper and had the same efficacy as ranibizumab[3–6].

Bevacizumab can trigger arterial thromboembolism because of exposure subendothelial procoagulant phospholipids by inhibiting VEGF endothelial regeneration and reducing the production of nitric oxide and prostacyclin[7–10].

Local intravitreal use of these agents is generally thought to be related to superior systemic safety since the drug is confined in the intraocular environment and the intravitreal dose is 400 times smaller than that used for intravenous treatment. However, it has been demonstrated that in spite of the existence of the blood ocular barrier, anti-VEGF agents injected into the vitreous might be detectable in plasma of treated patients[2].

Coagulopathy and hemostasis dysfunction are the main complications of hypertension. The increase in systolic and diastolic blood pressure is related to the disruption of coagulation factors11. Breakdown tunica intima of blood vessels in hypertensive patients causes atherosclerosis, thereby increasing platelet aggregation which triggers cardiovascular disease[12,13].

Hypercoagulation/prothrombotic status in hypertensive patients contributes to the risk of damage to target organs. This can be triggered by the activation of the renin-angiotensin system with abnormal platelet and endothelial function, coagulation, and fibrinolysis. Antihypertensive therapy targeted at the Renin-Angiotensin System (RAS) can produce reverse prothrombotic abnormalities that contribute to reducing thrombotic complications[14,15].

Prothrombin time (PT) and activated partial thromboplastin time (APTT) measurements can evaluate secondary hemostasis. Many studies suggest that a decrease in PT or APTT value is a risk factor for venous thromboembolism16. During monthly intravitreal anti-VEGF injection, coagulation parameters including PT and APTT are very important in patients with a history of cardiovascular and cerebrovascular disease because they can be a serious sign of thromboembolic events16. APTT shortening is a result of the accumulation of increased coagulation factors in the circulation due to increased in vivo coagulation activity[17].

METHODS

This study used a cohort design conducted at RSUP Dr. Sardjito in March 2019 - June 2019. The research protocol was approved by Universitas Gadjah Mada ethic committee (KE/FK/0226/EC/2019). The research subjects were interviewed using questionnaires, performed physical and ophthalmological examinations, then checked APTT value before IVB and one week (6-9 days) after IVB. The subjects of the study consisted of two groups, hypertensive and non-hypertensive. The hypertensive group is diabetic patients who are planned IVB with blood pressure at examination ≥140/90 mmHg and have a history of hypertension, both those who routinely consume antihypertensive drugs and those who did not routinely take antihypertensive drugs. The non-hypertensive group is diabetic patients who are planned IVB with blood pressure <140/90 mmHg and has never been diagnosed with hypertension by a doctor.

Inclusion criteria were patients aged 40-65 years, can be interviewed, willing to do laboratory tests and ready to check up on schedule. Exclusion criteria were patients who took anticoagulants (heparin, warfarin), had a history of coagulation disorders, and patients whose blood samples could not be tested.

APTT examination used the photopic method with the ACLTOP300 machine. Sample using 2cc venous blood then put in a 1:9 citrate tube. In less than two hours, the blood had to centrifuge for 10 minutes. The plasma was taken and put in the ACLTOP300 machine and APTT reagent (Calcium Chloride 0.02 M) was added. The APTT value will be measure photopically in seconds.

RESULTS

Table 1. Demographic and Clinical Characteristic Subjects

Variable	Hypertensive	Nonhypertensive	*p*
Age (year) (mean±SD)	55,78±5,14	53,97±7,06	0,255
Gender			
Male [n(%)]	13 (40,6)	19 (63,3)	0,074
Female [n(%)]	19 (59,4)	11 (36,7)	
Visual acuity (logMAR) (mean±SD)	0,50±1,64	1,02±0,89	0,126
BMI (mean±SD)	24,39±3,07	23,57±3,60	0,339
Dyslipidemia			
Yes [n(%)]	13 (40,6)	11 (39,3)	0,916
No [n(%)]	19 (59,4)	17 (60,7)	
Smoking			
Yes [n(%)]	4 (12,5)	0 (0)	0,114
No [n(%)]	28 (87,5)	30 (100)	
Activity score level			
Exercise/day [n(%)]	10 (31,2)	4 (14,3)	
Excercise 1-3x/week [n(%)]	6 (18,8)	6 (20)	0,228
Rarely [n(%)]	16 (50)	20 (66,7)	
Duration DM (year) (mean±SD)	9,5±6,49	10,17±7,46	0,827
Antidiabetic drug used			
Yes [n(%)]	30 (93,8)	29 (96,7)	0,286
No [n(%)]	2 (6,2)	1 (3,3)	
∑ IVB (mean±SD)	3±2,87	4,2±4,92	0,619
preIVB APTT value (s) (mean±SD)	36,47±2,92	36,33±4,39	0,464

SD = standard deviasion; BMI = Body Mass Index; DM = Diabetes Mellitus, IVB = Bevacizumab Intravitreal Injection

This study involved 66 subjects with 4 dropouts, 2 from the hypertensive group and 2 from the non-hypertensive group so there were 62 subjects, 32 hypertensive, and 30 non-hypertensive. Characteristics of the research subject are presented in Table 1 and no significant differences were found in the two groups.

In this study, shortening the APTT value after intravitreal bevacizumab injection has ensued in both of the groups, but the shortening value was not significantly different as in Table 2. In the hypertensive group, mean APTT value before IVB was 36.47 ± 2.92 seconds and after IVB was 35.42 ± 3.63 seconds with p value of 0.112, while in non-hypertensive group the APTT value before IVB was 36.33 ± 4.39 seconds and after IVB was 35.60 ± 3.13 seconds with p value of 0.214, so there was no significant difference in mean before and after IVB in both groups.

Table 2. APTT Value in Hypertension and Nonhypertension Groups

Variable	Hypertensive	Non-hypertensive	*p* Value
APTT preIVB (seconds) (mean±SD)	36,47±2,92	36,33±4,39	0,464
APTT postIVB (seconds) (mean±SD)	35,42±3,63	35,60±3,13	0,834
Δ APTT±SD (min-max)	-1,03±3,65 (-15,20 - (-3,4))	-0,73±2,55 (-8,90 – 4,20)	0,91

SD=standard deviasion; min=minimum range value; max=maximum range value

This study compared the percentage of patients who experienced shortening of APTT value in the hypertensive and non-hypertensive groups after the intravitreal bevacizumab injection procedure.

Table 3. Percentage of patients who had shortening APTT Value

	Shortened APTT (n,%)	Unshortened APTT (n,%)	RR (CI 95%)
Hypertension (n=32)	19 (59,4)	13 (40,6)	1,370
Nonhypertension (n=30)	13 (43,3)	17 (56,7)	(0,831-2,258)

RR= *Relative Risk*, CI= Confidence Interval

According to Table 3, there was no difference in the percentage of patients who experienced shortening of APTT value between the hypertensive and non-hypertensive groups (59.4% vs 43.3%) with RR 1,370 CI (0.831-2,258).

Table 4. Percentage Patients who had Shortening APTT in Hypertensive Group

	APTT shortening n(%)	Unshortened APTT n(%)	RR (CI 95%)
Hypertensive with antihypertensive drugs (n=23)	11 (47,8)	12 (52,2)	0,538
Hypertensive without antihypertensive drugs (n=9)	8 (88,9)	1 (11,1)	(0,331-0,874)

RR= *Relative Risk*, CI= Confidence Interval

In Table 4, it can be concluded that hypertensive patients who take antihypertensive drugs are protected from the risk of APTT shortening after intravitreal bevacizumab injection.

In this study there was an increase in intraocular pressure one week after intravitreal bevacizumab injection but not clinically significant and still within the normal range (mean IOP before injection 13.1 mmHg and one week after IVB to 13.7 mmHg) as shown in Figure 1.

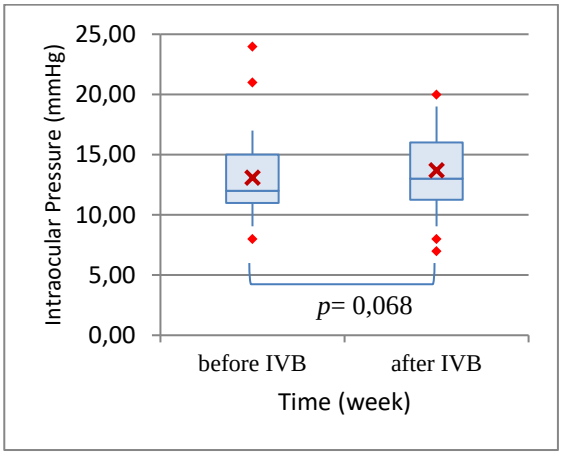

Figure 1. Intraocular Pressure Before and One Week After IVB in All Subjects

There were no differences in the systolic and diastolic pressure changes before and after IVB in both groups**Error! Reference source not found.** and Table 6.

Table 5. Systolic Blood Pressure Changes After IVB

Variable	Hypertensive (n=32)	Non-hypertensive (n=30)	*p* Value
Δ Sistolic (mean±SD)	-4,16±22,98	5,90±17,76	0,058
(Min-Max)	(-66-50)	(-27-54)	

Table 6. Diastolic Blood Pressure Changes after IVB

Variable	Hypertensive (n=32)	Nonhypertensive (n=30)	*P* Value
Δ Diastolik (mean±SD)	-2,91±13,48	2,2±9,98	0,165
(Min-Max)	(-40-20)	(-20-31)	

DISCUSSION

In this study, there is no significant difference in the APTT shortening value between hypertensive and non-hypertensive groups. APTT values between hypertensive and non-hypertensive groups are still controversial. This is caused by endothelial damage, platelet hyperactivity, and changes in coagulation as vascular complications of hypertension, so APTT value can be longer or shorter.

A longer APTT value in hypertensive patients can be caused by endothelial damage as a result of atherosclerosis caused by hypertension[11,12.] Jiskani et al evaluated the difference in coagulation parameters (PT, APTT, INR) between hypertensive and normotensive patients which resulted in a significant increase in coagulation parameters in the hypertension group rather than normotension[11]. However, this is contrast with the results of a study in China that found a shortening of APTT in patients with Pregnancy-Induced Hypertension Syndrome (PIH) compared to normal pregnancies which mean that pregnant patients with PIH are more hypercoagulable and at risk of thrombosis and secondary hyperfibrinolysis[18].

There was no difference in APTT values before and after IVB in the two groups as in Table 2. In 2014, study Zuohuizi et al reported that shortening of the APTT value was significant in the first week after IVR (Ranibizumab Intravitreal Injection). One theory suggests that VEGF barriers will reduce the production of nitric oxide and prostacyclin which causes increased blood viscosity and platelet aggregation[10]. Some researchers suggested that bevacizumab in damaging endothelial walls and producing subendothelial lipids so thrombus is formed[19]. Meanwhile, systemic VEGF inhibition by anti-VEGF agents was more demonstrated in neovascular AMD who received aflibercept intravitreal therapy compared with intravitreal bevacizumab, while ranibizumab did not affect systemic VEGF[20].

When compared with ranibizumab, the anti-VEGF agent approved by the FDA, it is known that the half-life of 1.25 mg IVB in the vitreous is 10 days, while the half-life of 0.5 mg IVR (Intravitreal Ranibizumab injection) in the vitreous is 3 days. The systemic concentration of bevacizumab after intravitreal injection of 1.25 mg ranged from 59.8 to 86.5 ng / mL, whereas injection of ranibizumab intravitreal 0.5 mg ranged from 0.3 to 2.36 ng / mL. However, regardless of the longer half-life of bevacizumab and higher concentrations after intravitreal administration, its ability to bind to VEGF is significantly lower (14-100times)[21].

Very low doses, intravitreal administration routes, and low bevacizumab affinity were thought to be the causes of shortening of APTT values that were not significant after IVB in this study. Table 3 shows that there is no increased risk of APTT shortening in hypertension. The correlation between the increased blood pressure and the risk of cardiovascular events is directly proportional.

Increasing in systolic 20 mmHg according to 2-3x cardiovascular events[22].

In most clinical cases, hypertension is often associated with blood hyperviscosity, but it is not clear whether this is the cause or effect of high blood pressure or previous treatment methods that affect this correlation. The latest hypothesis states that high blood hyperviscosity is not directly related to hypertension, but it is supported by genetic and/or environmental factors, including obesity, physical activity, and smoking habits[23]. This supports the results of this study, that mean of APTT between hypertensive and non-hypertensive patients was not different either before or after IVB.

Using antihypertensive drugs regularly can protect from the risk of shortening APTT as shown in Table 4. Essential antihypertensive treatment targeted at RAS can produce reversal prothrombotic and contribute to a reduction of thrombosis related complications. Several studies have shown the beneficial effects of ACE inhibitors and ARBs (Angiotensin II Receptor Blocker) on prothrombotic conditions in addition to their efficacy in reducing blood pressure. Antithrombotic effects that have the potential to inhibit RAS can support cardiovascular function[14].

In Figure 1, there was no significant difference in intraocular pressure before and one week after IVB, and this was still within the normal range. In 2016, Lee et al reported that an increase in intraocular pressure occurred immediately after intravitreal injection and returned to normal in 30 minutes[24].

In Table and Table 6, there were no significant differences in changes in systolic or diastolic pressure before and one week after IVB in both groups. Study Rasier et al in 2009 found an increase in systolic and diastolic one week after intravitreal bevacizumab injection in both the hypertension group and in the non-hypertensive group, but these changes were not clinically significant[25].

Al-Dross and Quabain also examined changes in blood pressure in controlled hypertension and normotension after IVB but the results did not change significantly so IVB was safe for patients with controlled hypertension[26]. Anti-VEGF can damage vascular homeostasis and physiological responses to stress, with pathological consequences such as disorders of wound healing, hypertension, arterial thromboembolism, cardiac dysfunction, and renal toxic[27].

In the phase II study, the use of intravenous bevacizumab in colorectal cancer caused hypertension complications. There was 8.5% in the 5mg / kgBB group and 25% in the 10mg / kgBB group who had grade 3/4 hypertension complications. The mechanism of hypertension caused by the use of anti-VEGF agents is associated with VEGF inhibition at endothelial level[28]. In this study, the intravitreal dose of bevacizumab given was much smaller than the intravenous, so there were no significant changes in systolic and diastolic pressure in the two groups.

The limitation of this study was subjects with comorbidities were included in this study. The presence of comorbidities can cause changes in coagulation parameters.

CONCLUSION

There was no difference in the shortening of APTT value one week after intravitreal bevacizumab injection between hypertensive and non-hypertensive groups in patients with diabetes mellitus. Hence, the administration of IVB in hypertensive patients with regular antihypertensive therapy might be safe.

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