Original Research Article

EVALUATION OF INTRAVITREAL BEVACIZUMAB FOR ITS SYSTEMIC SIDE EFFECT THROMBOSIS AFTER ACUTE ADMINISTRATION

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ABSTRACT

Bevacizumab is a humanized monoclonal antibody that targets Vascular endothelial growth factor-A (VEGF-A), an isoform of Vascular endothelial growth factor(VEGF) thatstimulates endothelial cell proliferation and subsequent migration. Bevacizumab specifically binds to the VEGF-A protein, thereby inhibiting the process of angiogenesis. As thrombosisand hypertension are the major systemic side effects of bevacizumab whether this drug couldpredispose a patient to thrombosis or not after intravitreal administration, as it is absorbed even through intravitreal administration, we determined the safety of the drug. For this purposetotal 10 patients were selected and followed for acute effects and 10 patients were administered three doses of intravitrealbevacizumab at monthlyinterval and followed for chronic effects of drug. Blood samples were taken to determine fibrinogen level, platelet count,prothrombin time (PT), activated partial thromboplastin time (APTT) andsodium level by kit method. Blood pressure was also monitored of all the patients before and after the drug administration. There has been non-significant decrease in the fibrinogen levels in acute stage. Non-significant rise in the PT.Platelet counts decrease insignificantly. No significant change is observed in sodium levels after the injection in acute stage.Slight increase is noted in diastolic blood pressure where as slight decrease is noted in systolic blood pressure.

***Keywords*:** Bevacizumab, thrombosis, intravitreal, acute.

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INTRODUCTION

Neovascularization of the choroidaland retinal tissue are the leading cause of blindness in developed countries [1]. Vascular endothelial growth factor(VEGF) has been identified as a major angiogenic stimulus in the variety of retinal and choroidalneovascularia tion[2].

Vascular endothelial growth factor(VEGF) is homodimeric glycoprotein and is a growth factor specific or endothelial cells [3]. Not only it promotes growth and survival of vascular endothelial cells, but it also causes conformational changes of tight junctions of retinal vascular endothelial cells leading to increased vascular permeability [4].

Advances in our understanding of pathogenesis of Choroidal and retinal neovascularization have facilitated the development of drugs specifically directed against Vascular endothelial growth factor.

Bevacizumab (Avastin) is a humanized monoclonal antibody to Vascular endothelial growth factor approved for the treatment of colorectal cancer. It has been used systemically and intravitreally for the

treatment of neovascularchoroidal and retinal diseases since july 2005. Significant improvements in visual acuity were seen.

Intravitrealbevacizumab has been described in some recent articles for treatment of proliferative diabetic retinopathy complicated byvitreous hemorrhage [5], macular edema in central retinal vein occlusion, and neovascular age-related macular degeneration [6].

The permeability inducing actions of VEGF appear to induce the development of macular edema. VEGF may cause macular edema that accompanies diabetic retinopathy, retinal vein occlusion and uveitis. Human eyes with macular edema secondary to uveitis, retinal vein accession and diabetic retinopathy have shown increased retinal levels of VEGF [7]. When VEGF was blocked, blood-retinal barrier breakdown could be both prevented and reversed through VEGF inhibition.

Angiogenesis is an essential process in tumor development [8]. The VEGF ligand is the predominant regulator of tumor angiogenesis [9]. Avastin directly targets the VEGF ligand to specifically inhibit angiogenesis [10].Maintaining VEGF ligand inhibition may prevent tumor vessel regrowth over time [11].

Bevacizumb is recognized to be associated with an increased risk of arterial thromboembolic events [12] which may result from the anti VEGF effect and also it may reduce the production of nitric oxide and prostacyclin, thus predisposing to thromboembolic events [13].

As thrombosis and hypertension are the major systemic side effects of bevacizumab whether this drug couldpredispose a patient to thrombosis or not after intravitreal administration, as it is absorbed even through intravitreal administration. We determined the safety of the drug and we also determined whether acute dosing is safer as compared to chronic dosing.

Since no work has been carried out before on this issue in our local setup, this study would be important in decision making regarding the safety of intravitrealbevacizumab in choroidal and retinal neovascular disorders.

MATERIAL AND METHODS

Inorder to evaluate the systemic side effects of intravitrealbevacizumab total 10 patients were selected. Patients were selected from Al Ibrahim eye hospital, meeting the inclusion and exclusion criteria receiving Bevacizumab (Avastin) injection. The purpose, procedure, risks and benefits of the study was explained to the patients. Informed consent was taken. In operation theater before injecting the inravitrealbevacizumab,eye ball was anesthetized with topical proparacaine drops sterilized with povidone iodine 5%.

Inclusion criteria:

Patients having neovascularization in retina and choroid of eye like diabetic retinopathy and age related macular degeneration diseases including both males and females having age above 40years. Hypertensive and non hypertensive,diabetic and non diabetic patients were included.

Exclusion criteria:

Patients having any hematological disorders like thrombosis.

History of previous Bevacizumab injection.

Drug:

Patients received the drug Bevacizumab. This drug wasintravitrealy administered by Professor Dr.P.S.Mahar.

The dose of intravitrealbecizumab administered to the patient is 1.25mg in 0.05ml with 1ml syringe.

Experimental protocol:

These 10 patients were administered single dose of intravitrealbevacizumab and followed for acute effects. Blood samples were taken before and after one week of drug administration to determine fibrinogen level, platelet count,prothrombin time (PT), activated partial thromboplastin time (APTT) andsodium level by kit method. Blood pressure was also monitored of all the patients before and after the drug administration.

Sample collection:

Blood samples were collected in three types of tubes:

BD vacutainer EDTA.K2 tubes for collection of pure blood to study hematological parameter like platelet count.

Bio vac 3.2% sodium citrate tubes for collection of plasma to study coagulation parameters like PT and APTT and fibrinogen level. Prothrombin timeand activated partial prothrombin time was checked manually using standard reagent kits of DiagnosticaStago France.

B-ject Gel clot activator vaccum tube for collection of serum to study sodium level.

After the collection of samples 3 ml of blood in 3.2% sodium citrate tubes, plasma was separated out and 4 ml of blood in Gel tubes, Serum was separated out, by centrifuging the blood samples in 800 centrifuge machine (China) at 4000 RPM for 5 minutes. The separated plasma and serum was stored in 2-8℃ and within 3 hours all the coagulation and electrolyte estimation were performed.

The estimation of platelet was directly done on automated MS4E Vet Hematology analyzer model# 3MSR0214 by MeletSchloesing Switzerland.

After separating the plasma the fibrinogen level was measured by humaclot duo (coagulation analyzer, model# 18650) (Human Germany) using standard reagent kits of Human Germany. Hemostat fibrinogen (manual and automated determination of plasma fibrinogen) was used [14].

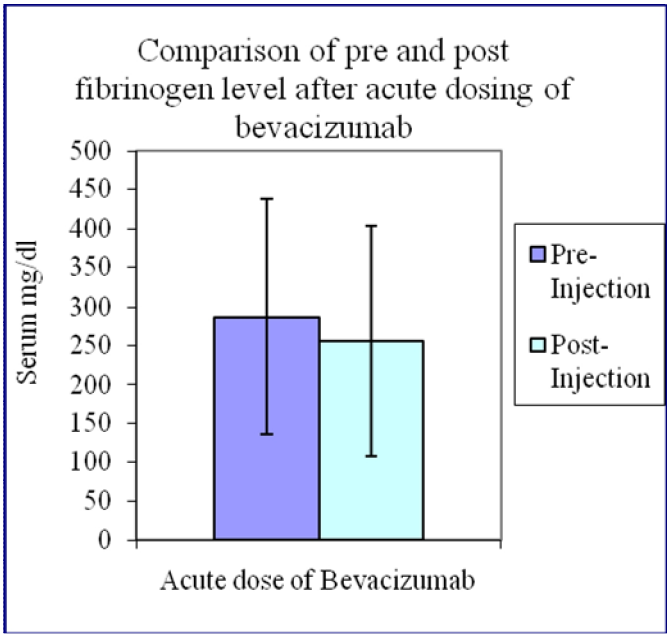
Neoplastin CI Plus was used for the determination of prothrombin time [15].C.K.Prest was used for the determination of Kaolin-activated partial thromboplastin time [16,17].

After separating the serum, the sodium level was measured by Microlab 300 semi-automated clinical chemistry analyzer by Merck Netherlands.

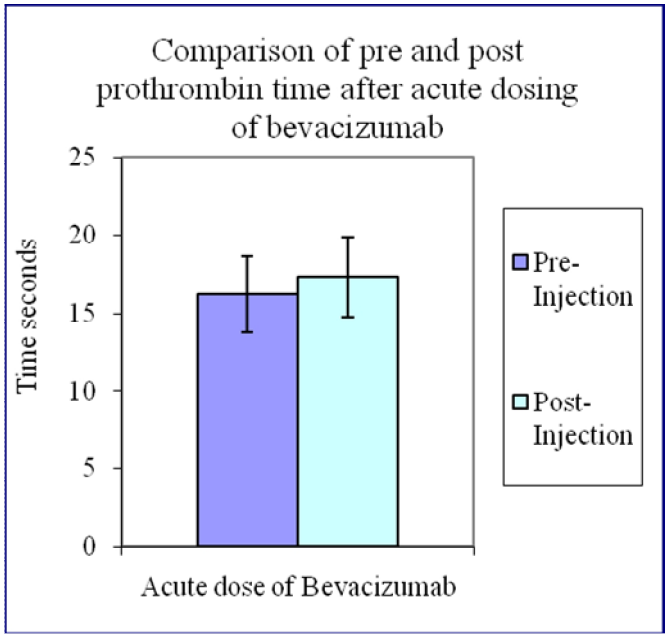
RESULT

There is increasing evidence that medications targetting VEGF are effective in the treatment of choroidal neovascularization associated with age related macular degeneration. Bevacizumab targets Vascular endothelial growth factor-A. Scutz*et al* in the study reported that Bevacizumab treatment is associated with significant increase in the risk of arterial thrombosis [18]. In our present study we examined blood parameters like platelet count, fibrinogen level, prothrombin time (PT), activated partial thromboplastin time (APTT) to evaluate whether intravitrealbevacizumab may cause thrombosis or not. Sodium level was also conducted by kit method. Blood pressure was also monitored of all the patients before and after the drug

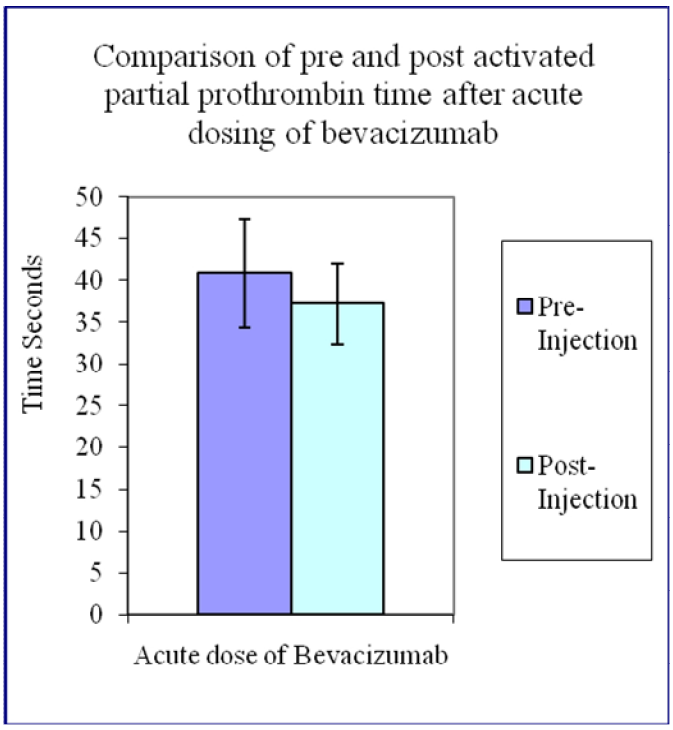
GRAPH-1:Effect of Bevacizumab on fibrinogen level



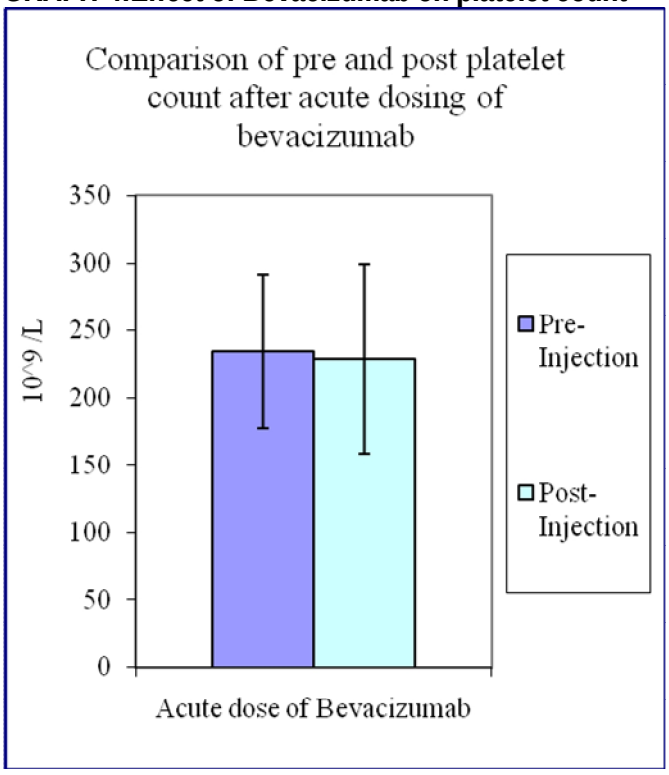
GRAPH-2:Effect of Bevacizumab on prothrombin time



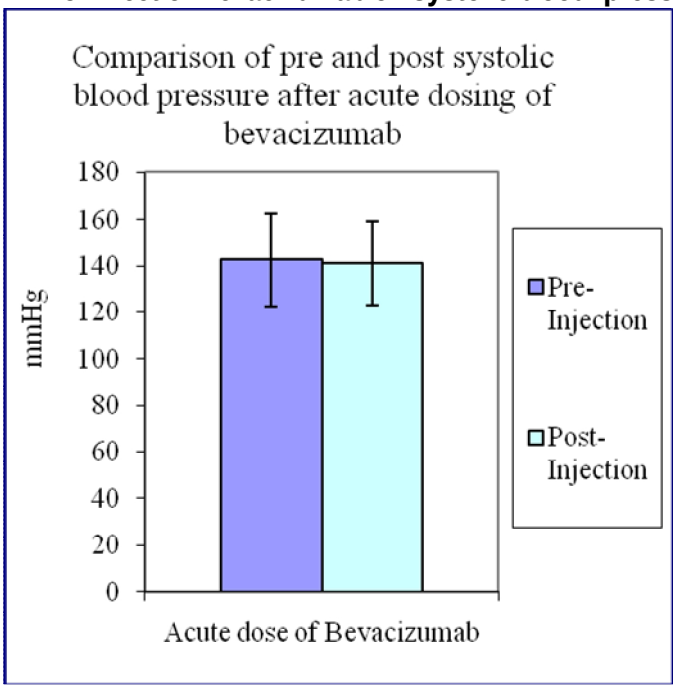
GRAPH-3:Effect of Bevacizumab on aPTT



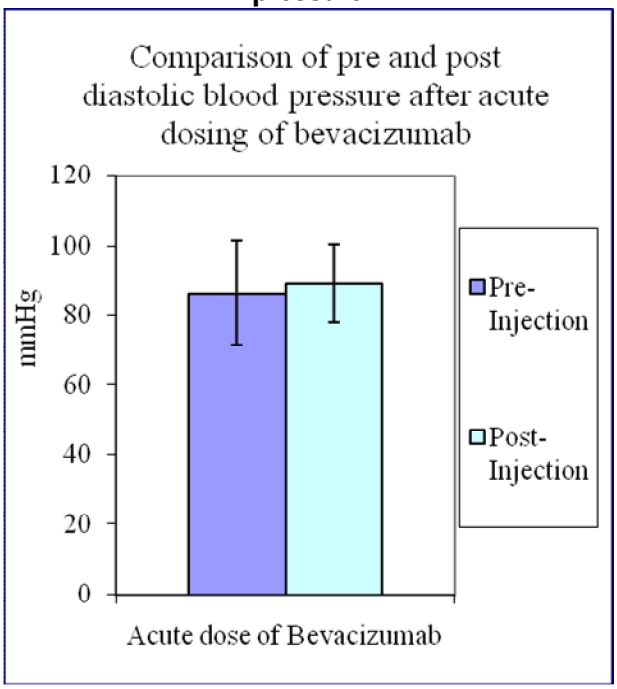
GRAPH-4:Effect of Bevacizumab on platelet count



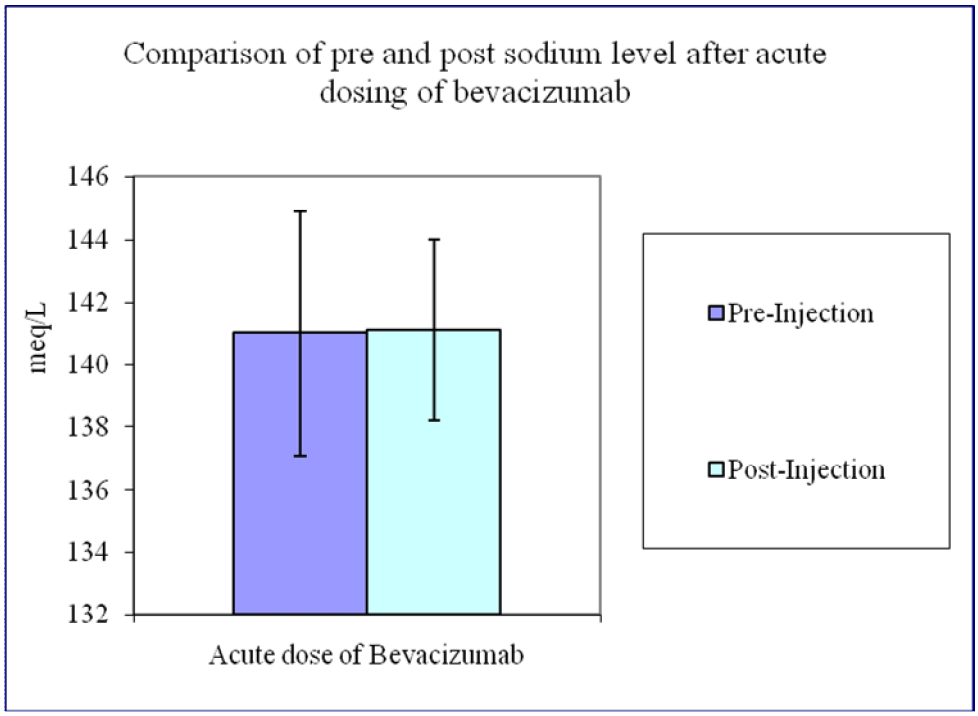
GRAPH-5:Effect of Bevacizumab on systolic blood pressure



GRAPH-6:Effect of Bevacizumab on diastolic blood pressure



GRAPH-7:Effect of Bevacizumab on sodium level



DISCUSSION

There has been some rise in the PT in acute stage but it is non-significant. Decreased level of fibrinogen and platelet count may be causing this rise of PT. The results of our study indicate that there is slight risk of bleeding after using intravitrealbevacizumab in neovascularization of choroid and retina. APTT is non-significantly decreased after the injection in acute treatment.

Platelet counts decrease after the injection but this decrease is also insignificant. One physiological function of platelets may be to act as scavangers of circulating VEGF to restrict angiogenic activity to sites of wound healing. This may also be occurring in the pathological situation of neovascularization [20]

No significant change is observed in sodium levels after the injection in acute stage.

Slight increase is noted in diastolic blood pressure while systolic blood pressure is slightly decreased in acute stage but it is insignificant. Raiser *et al* study supports our results, reporting that there is a risk of dysregualtion of blood pressure or persistence of hypertension in hypertensive patients after intravitrealbevacizumab injections [21].

Mourad*et al*. reported that bevacizumab treatment resulted in endothelial dysfunction and capillary rarefaction; both changes are closely associated and could be responsible for the rise in blood pressure observed in most patients [22].

The results of our study indicate that there may be bleeding tendency after bevacizumab because fibrinogen level and platelet counts have decreased after acute administration so careful monitoring is required in patients receiving this drug, as well as monitoring of blood pressure is required. If patient have any history of bleeding disorder or cardiovascular disease then acute dosing of bevacizumab may worsen the situation. In our study intravitreal administration ofbevacizumab produced a slight decrease in systolic and slight increase in diastolic blood pressure so a hypertensive patient may receive acute dosing of intravitrealbevacizumab.

FURTHER EXTENDABLE WORK

This work could be further extended to reassess the results with an increased sample size and also determine the effects in complicated patients having secondary problems.

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