Evaluation of Changes in Bleeding Time in Hypertensive Patients on Amlodipine - A Prospective Observational Study

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ABSTRACT

Background: Platelet hyperactivity is a risk factor in hypertensive patients that paves the way to atherothrombosis causing cardiovascular and cerebrovascular events. Counteracting platelet aggregation is an established step in preventing thrombotic events. Calcium channel blockers are one among the recommended antihypertensive agents according to 2013 ESH/ESC (European Society of Hypertension and European Society of Cardiology) guidelines. In addition to lowering blood pressure they are known to have antiplatelet activity. Dihydropyridine class of L-type calcium channel blockers are the most potent group and shares this activity. Anti-platelet aggregatory effect of these agents could supplement its anti-hypertensive property and could prove to be desirable in the treatment of hypertensives with high risk of atherosclerotic and thromboembolic risk. So the present study aims to explore the anti-platelet activity of Amlodipine. **Objectives:** To evaluate the anti-platelet activity of Amlodipine in hypertensive patients. **Methods and Materials:** Sixty subjects were enrolled in the study. Test group comprised of thirty patients with essential hypertension, who were prescribed Tab Amlodipine at doses 5 mg or 10 mg once daily orally. Patients included in study group were regularly on Amlodipine for at least one month. 30 normotensive subjects, who are not on any drug affecting platelet function like Aspirin, dipyridamole, statins etc. acted as control group.Duke method of Bleeding time estimation was used to assess changes in Bleeding time. **Statistical analysis:** Student's unpaired *t*-test was used to compare Amlodipine test group with the normotensive control group. Results were expressed as Mean bleeding time ± SEM. SPSS software version 20 was used for statistical calculations. **Results:** The mean bleeding time of Amlodipine group was 2.214 minutes ± 0.028 SEM. The control group had a mean bleeding time of 1.998 minutes ± 0.036 SEM. The result gave a statistically significant *p* value of <0.001. Average duration of treatment was 3.683 years. **Conclusion:** The statistically significant bleeding time observed in Amlodipine group suggest that it has anti-platelet activity.

Key words: Amlodipine, Hypertension, Bleeding time, Antiplatelet activity.

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INTRODUCTION

Hypertension is one of the leading causes of global disease burden.[1] The global prevalence of elevated blood pressure in age groups more than 25 years was 40% in 2008.[2] Nearly 1 billion adults had hypertension in 2000 and it is estimated to rise up to 1.56 billion by 2025.[3] Most of the clinical events related to hypertension is due to atherosclerosis and thrombosis.[4] Elevated arterial pressure causes pathological changes in vasculature[5] and activated platelets contributes to the vascular damage. This platelet hyperactivity acts as a

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risk factor in hypertensive patients, causing atherosclerosis and atherothrombosis leading to cardiovascular and cerebrovascular events.[4] Treatment with antiplatelet agents is a crucial step in preventing thrombotic events.[6] Calcium channel blockers are one among the recommended antihypertensive agents according to 2013 ESH/ESC (European Society of Hypertension and European Society of Cardiology) guidelines, used as either single agent or in combination. They are associated with fewer adverse effects.[7,8] Dihydropyridine class of L-type calcium channel blockers like Amlodipine, the most potent group among the calcium channel antagonists have antiplatelet activity.[8] Anti-platelet aggregatory effect of these agents in addition to anti-hypertensive property could prove to be desirable in the treatment of hypertensives with high atherosclerotic and thromboembolic risk.[9] So the present study aims to explore the anti-platelet activity of Amlodipine.

Bleeding time can be a potential indicator of platelet activity.[10,11] So an increase in bleeding time can be used as a marker of anti-platelet aggregatory action. Duke method of Bleeding time estimation is a laboratory test that can be used for estimating anti-platelet activity and is still in use as an investigational test.[11] The advantages of Duke method is that it is economical, easy to perform, less invasive and results are obtained quickly.[12] However it has certain disadvantages like difficult to reproduce and to standardize. Another method called Ivy's method when used with a template could solve these disadvantages but is not so acceptable for the subjects as it involves a long incision of 10 mm × 2 mm made on forearm. Light transmission aggregometry is the Gold standard for platelet aggregation tests.[13] This instrument is available only in few institutes.

MATERIALS AND METHOD

A total of (n=60) subjects were enrolled in the study. Test group comprised of (n=30) patients with essential hypertension, who were prescribed Tab Amlodipine 5 mg or 10 mg once dialy orally. Thirty (n=30) normotensive subjects acted as control group.Initial baseline characteristics like SBP, DBP, age, sex, duration of treatment and average dose of drug used were recorded. Duke method of Bleeding time estimation was used to assess changes in Bleeding time in both the groups. Subjects included in study were ≥18 years of age and of both sexes. Study group were patients diagnosed with essential hypertension and regularly on Amlodipine for at least one month. Patients with secondary hypertension, suffering from fever, comorbid bleeding disorders, pregnant and lactating women were excluded from the study. It was also ensured that subjects of both groups were not on any medication altering platelet function like Aspirin, dipyridamole, statins etc.

Duke method of Bleeding time estimation was done for both the groups. One Staff in the lab was trained for performing the test and recorded all the results. Lancet was used to prick left fingertip and a filter paper was used to wipe the blood every 15 seconds till the bleeding stops.[10,11] The test was conducted twice in each subject and on finding a difference in observed value, mean was taken as the bleeding time.

RESULTS

The baseline characteristics of both the groups are given in Table 1. As mentioned in Table 2, the mean bleeding time of Amlodipine group was found out to be 2.214 minutes ± 0.028 SEM. The control group recorded a mean bleeding time of 1.998 minutes ± 0.036 SEM (Represented in Figure 1). Student's unpaired *t* test gave a statistically significant *p* value of <0.001. Average duration of treatment was 3.68 years. Average dose of Amlodipine was 6.66 mg/day.

DISCUSSION

The study shows that Amlodipine group showed a statistically significant increase in bleeding time. This increase in bleeding time can be interpreted as alteration of platelet function by Amlodipine possibly by antiplatelet aggregatory activity. Amlodipine belongs to Dihydropyridine class of L-type of calcium channel antagonists. They are known to possess antiplatelet activity. The exact molecular mechanism remains unclear as platelets do not possess L-type calcium channels. But it is known that platelet intracellular calcium plays a crucial role in platelet aggregation. Activation of NO/cGMP-dependant signalling pathway has been proposed as a possible mechanism of anti-plateletactivity.[8] Through phosphorylation of endothelium derived Nitric Oxide Synthase eNOS through Protein Kinase C (PKC) pathway, Amlodipine can enhance its functions.[14] NO can activate guanylylcyclase, calalyzing conversion of GTP to second messenger cGMP.[15] Soluble Guanylylcyclase (sGC) is a receptor for NO. There are two isoforms of sGC –β1α1 and β1α2 both of which has same function and translocate from cell cytosol to plasma membrane after activation due to heat shock protein 70 and possibly due to Ca^{2+}, Mg^{2+} and ATP and GTP. cGMP dependant protein Kinase1 (PKG1) is associated with platelet activity and is present in higher concentrations in platelets than in vascular endothelium. Three types of PKG exists: cytosolic PKG with 2 isoforms

Table 1: Baseline characteristics of subjects

	Test Group (Amlodipine)	Control Group
Age (in years)	54.8	51.933
Sex		
Males	16	15
Females	14	15
SBP	130	121.667
DBP	84.667	83.333
Average Dose (mg/day)	6.66	-
Average Duration of treatment (in years)	3.683	-

Table 2: Mean bleeding time (BT) ± SEM of Amlodipine and Control

	Mean BT*	SEM
Test Group	2.214	0.028
Control Group	1.998	0.036

*Mean BT in minutes.
Statistically significant *p* value<0.001.

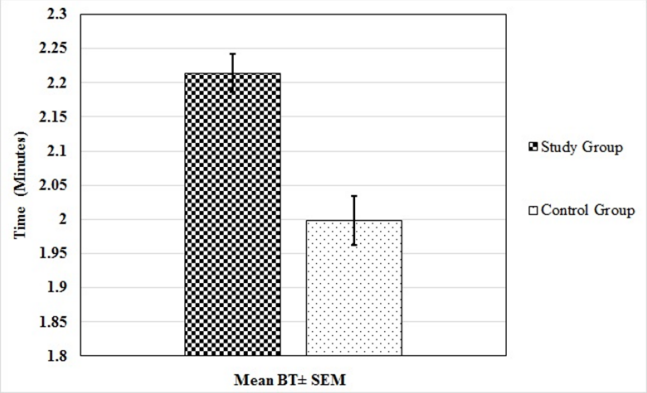

Figure 1: Bar diagram representing Mean BT ± SEM of Amlodipine and Control.

PKG1α and PKGβ and membrane bound PKG2. PKG1β is predominantly present in platelets.[8,16] NO mediated increase in cGMP inhibits platelet aggregation.[17] Increased up-regulation of NO/cGMP/PKG1 cascade inhibits platelet activity through following mechanisms: regulation of actin filament dynamics, intra cellular Ca^{2+} mobilization and activation of integrin. These mechanisms will in turn suppress Phospholipase C and Protein kinase C activity. PKG1 promotes sarcoplasmic reticulum ATPase (SERCA)- dependant refilling of intra platelet Ca^{2+} stores and inhibits IP3 (Inositol (1,4,5-tris phosphate) stimulated Ca^{2+} release from sarcoplasmic reticulum. This results in decreased intracellular Ca^{2+} levels in platelets and decreases platelet activation. Further PKG phosphorylates TXA2 receptor and inhibits its function.[8,16]

Another study suggests that Amlodipine possess additional PPAR β/δ agonistic activity.[18] Even though platelets do not have a nucleus, they contain transcription factors like PPAR (Peroxisome Proliferator-Activated receptor and NF-ϰB). It has been found that platelets have three isoforms of PPAR: α, β/δ and γ. Activation of PPARs inhibit platelet activation through non genomic mechanism and additionally slows down the progression of intra arterial thrombus formation by increased NOS expression. Nifedipine is dual PPARβ and PPARγ activator. These property is unique to Amlodipine and Nifedipine and is not shared by other calcium channel antagonists.[18] In this study we have used Duke method of bleeding time estimation and we found statistically significant increase in BT which indicates its antiplatelet effect and it supports previous studies which has shown similar effect.[19]

CONCLUSION

Amlodipine has anti-platelet activity. Increase in mean bleeding time in Amlodipine group when compared with that of control group seen in results of our study is a reflection of anti aggregatory activity of platelets. We recommend more studies of larger scale on antiplatelet activity of amlodipine with a larger sample size and at various doses, be carried out as it is beneficial to reduce the overall morbidity and mortality in hypertensive patients with cardiovascular disorders. Its implications on drug interactions with other antiplatelet drugs should be worked out.

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