Original article:

Resistance to Aspirin and Clopidogrel among patients with Diabetes Mellitus: A cross sectional study from eastern India

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Abstract:

Objective : Diabetes mellitus is characterized by a prothrombotic state that is caused by many factors , one of which is platelet hyperreactivity.Antiplatelet therapy with Aspirin and Clopidogrel has emerged as critical therapy for both the primary and secondary prevention of coronary artery disease, but variability in the response to these drugs is well established. These patients have been categorized as responders, semiresponders and nonresponders. In this study we have attempted to assess the prevalence of Aspirin and Clopidogrel resistance by light transmission aggregometry (LTA) in patients with diabetes mellitus at a tertiary care centre in eastern India.

Material and method : This is an observational cross sectional study conducted from July 2015 till April 2017. Fifty two diabetic patients with high risk disease were prescribed Aspirin or Clopidogrel each of 75 mg/day. LTA was performed as baseline as well as post Aspirin/Clopidogrel administration.

Result : For Aspirin,44.2% patients were responders, 40.4% semiresponders and 7.7% were resistant. Among seven patients tested for Clopidogrel three each were responders and semiresponders, while one was resistant. Among the various parameters studied, only difference in serum creatinine was found to be statistically significant among three groups (of Aspirin responders, semiresponders and resistant)

Conclusion : The prevalence of antiplatelet drug resistance in eastern India is similar to that reported by other studies. Aspirin can be initiated for most patients. Clopidogrel can be used as a second line agent in case of contraindication to Aspirin or adverse effects due to the latter. The minimum dose has been used for both the drugs. Thus the cost of therapy can be lowered, benefitting a huge population However, the small study size, lack of follow-up or variation of drug dose are the limitations of the study.

Keywords: Diabetes mellitus, Aspirin, Clopidogrel, resistance to antiplatelet agents

Introduction:

Diabetes mellitus is the commonest endocrine disorder worldwide. The disease has many complications, and a major cause of mortality and morbidity in our country is coronary artery disease. The prothrombotic state that is present in diabetic patients is caused due to many factors, viz. endothelial dysfunction, increased coagulation, impaired fibrinolysis and platelet hyperactivity (1). The latter is important as hyperreactive platelets may contribute to the higher proportion of diabetic patients with inadequate response to antiplatelet agents compared to nondiabetic subjects. Antiplatelet therapy with Aspirin and Clopidogrel has emerged as critical therapy in both the primary and secondary prevention of coronary artery disease . Inspite of this recurrent atherothrombotic events occur in some patients on continued antiplatelet therapy(1,2,3,4)

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Variability in the response to Aspirin and Clopidogrel therapy has been well established(5). Nonresponsiveness or resistance to antiplatelet therapy has been studied in recent years by a number of laboratory methods(6,7,8). Criteria have been set to define responders, semiresponders and nonresponders(9,10)

Aims and Objectives

In this study we have attempted to assess the prevalence of Aspirin and Clopidogrel resistance by light transmission aggregometry in patients with diabetes mellitus at a tertiary care centre from eastern India.

Material and method:

This is an observational cross sectional hospital based study conducted at a tertiary care centre of eastern India. This study was conducted from July2015 till April 2017. Patients of all age from outpatient department of Hematology and endocrinology were included after proper screening.

Inclusion criteria: Patients being treated for Diabetes Mellitus who will be prescribed either Aspirin or Clopidogrel each of 75 mg per day. Such high risk patients include: Male or Female ≥50 year age, hypertension, dyslipidemia, acute coronary syndrome in patient or first degree relative. Exclusion criteria: concurrent use of other antithrombotic or nonsteroidal anti-inflammatory drugs, family or personal history of bleeding disorder, patients with known primary haematological disorder or malignancy , undergoing regular dialysis, platelet count< 1 lac/cumm and alcohol or drug abuse.

Sample size: Fifty two subjects fulfilling the above criteria

Method of data collection: Patients were enrolled into study either directly from Endocrinology OPD or when patients were referred to the Hematology OPD for evaluation of anemia . For all patients entering study a questionnaire including demographic information was completed.

Study design: Following the baseline LTA patients were advised either Aspirin 75 mg/ day or Clopidogrel at the same dose. The latter was advised in case of known peptic ulcer disease or intolerance to aspirin. Repeat LTA was performed 7 days after Aspirin intake or 4 hrs after Clopidogrel intake.

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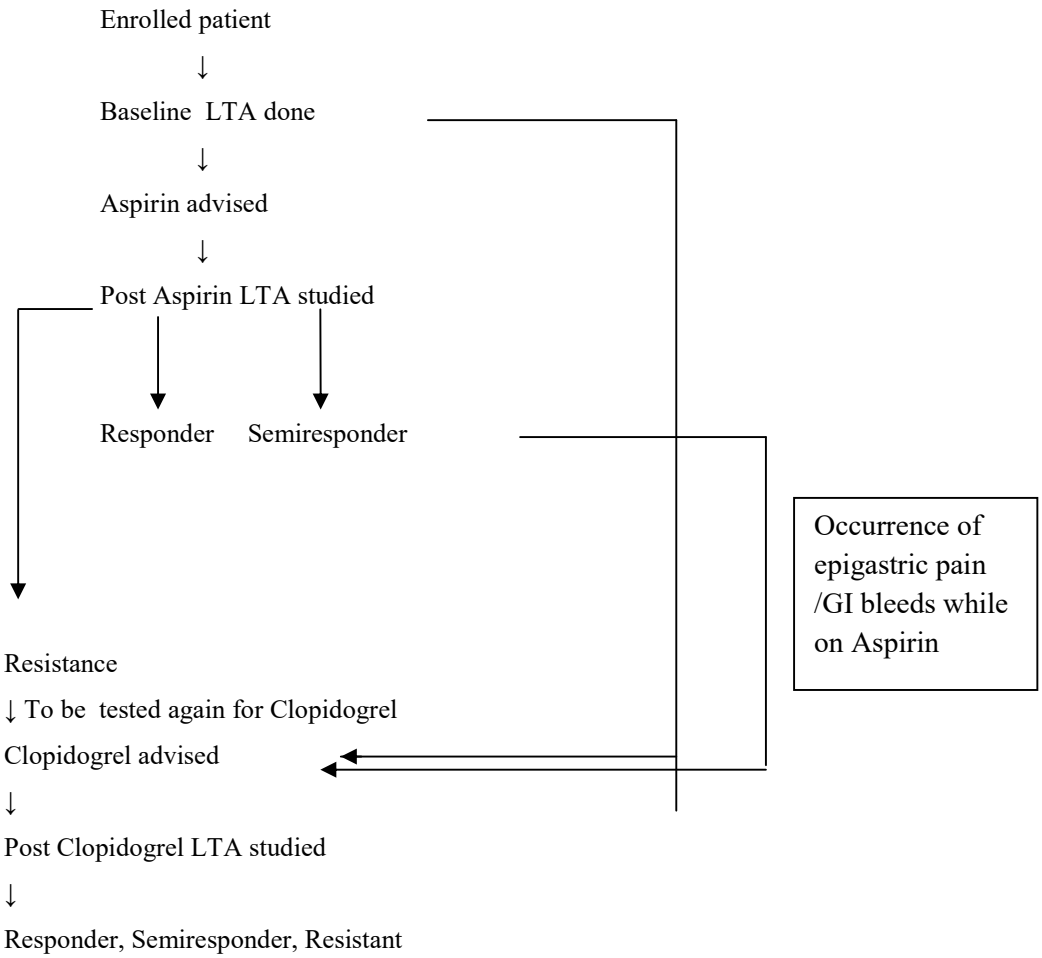
Plan of study



Platelet aggregation study was done with *reagents* Adenosine diphosphate (ADP) 10 µM

ADP 5 µM

Arachidonic acid (AA)0.5 mM

(Chronopar, Chrono-log corp,Havertown, PA 19083)

Instrument used : Dual channel chronolog model 700.

Procedure: After the patient has been comfortably seated for for about 20-30 minutes, by clean venepuncture using 21G needle, 5 ml blood was collected into 3.2% Na citrate(BD) Blood to anticoagulant ratio was 9:1. LTA was tested within 3 hrs of sample collection.

Platelet rich plasma was prepared by centrifuging blood at room temperature at 1200 -1300 rpm for 10 minutes. 250 µL of . Platelet rich plasma thus prepared was dispensed into each of three cuvettes where bud stirrers have already been placed.

The citrated blood was again centrifuged at higher spin (4000 rpm for 15 minutes) to obtain platelet poor plasma. 500 µL of platelet poor plasma was then dispensed into a separate cuvette.

Calibration done by one cuvette with PRP=0% and another cuvette with PPP=100% transmission. These cuvettes were preincubated at 37^{o}C for three minutes before addition of agonists.

In the meantime , agonists have been brought out from the respective refrigerators,(-70^{o}C for Arachidonic acid, 2-8^{o}C for ADP) and brought to room temperature. AA was vortexed before addition.

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Aggregation was observed for a four minute period.

In case of two patients , both AA and ADP caused a baseline aggregation of less than 50%, though they had no bleeding symptoms. Hence they were excluded from the study.

Aspirin resistance was defined as a mean aggregation of ≥70% with 10 μM ADP and mean aggregation of ≥20% with 0.5 mg/ml Arachidonic acid. Semiresponders are defined as those meeting only one criteria.

Clopidogrel resistance was defined as less than 10% decrease from baseline in platelet aggregation . Clopidogrel semiresponse is defined as 10-29% (<30%) decrease from baseline in platelet aggregation. Response is >30% decrease from baseline. Nonresponse is defined as composite of Resistance and semiresponders.

The aggregation curves were stored in JPEG format to be retrieved at a later date.

A masterchart was made in excel datasheet to include patient demographics, biochemical parameters, and aggregation test results. Statistical analysis was done by EPI-INFO,version 3.4.3and significance of difference between the three groups (Responder, Semiresponder and Resistant) was noted by the ANOVA test.

Results:

Among the 52 patients analysed in this study, 35 patients were males. 42 patients were hypertensive. Three patients had history of acute coronary syndrome (ACS). Among them two patients had both ACS and hypertension. 23(44.2%) patients were responders to Aspirin, 21(40.4%) patients were semiresponders and four (7.7%)were Aspirin resistant.

In case of Aspirin responders, the baseline platelet aggregation with AA was 60 -100%. The baseline aggregation was 50-100% for ADP with both 5mM and 10 mM doses. Following Aspirin administration the platelet aggregation with AA was by definition less than 20% and that with ADP was 0- 65% . In the group of patients who were semiresponders to Aspirin , the baseline platelet aggregation with both AA and ADP (10mM) was in the range of 60-100% . Following Aspirin administration , with AA the range of aggregation was 0-50% and that with 10mM ADP was 0-90%. Among the Aspirin resistant patients the baseline response to AA and ADP showed minimum change (70-100% pre Aspirin vs 40-90% post Aspirin).

Similarly platelet aggregation after Clopidogrel administration was analysed. In three patients who were Clopidogrel responders , though the baseline response with ADP (5mM and 10 mM)was in the range of 60-80% after Clopidogrel administration the aggregation fell to less than 35%. In the lone patient who was Copidogrel resistant , her baseline value of 80% aggregation with 10mM ADP was unchanged after Clopidogrel administration. The three patients who were semiresponders showed a drop in aggregation by 10-15% from the baseline after administration of Clopidogrel. Among the various parameters analysed in this study, viz age, duration of diabetes and hypertension, HbA1c, LDL cholesterol, triglyceride, baseline aggregation with ADP (5mM, 10mM), only difference in serum creatinine was found to be statistically significant among three groups (Aspirin responders, semiresponders and resistant)

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Table 1.Difference in serum creatinine is statistically significant in the three groups

Parameter	Asp Responder Mean(std dev)	Asp semiresp Mean(std dev)	Asp resistant Mean(std dev)	P value
age	57.22(8.63)	57.86(9.69)	63.00(9.35)	0.511
Duration of DM	6.78(3.48)	7.75(7.11)	5.75(3.69)	0.733
Duration of Hypertension	5.68(3.63)	5.76(5.34)	4(0)	0.931
HbA1c	6.92(0.84)	6.84(.69)	6.52(1.11)	0.665
LDLc	152(44.32)	141.52(34.95)	135.75(31.62)	0.591
TG	205.26(57.29)	220.33(53.07)	187.00(59.36)	0.461
Serum creatinine	1.04(0.18)	1.17(0.13)	1.17(0.31)	**0.048**
Baseline AA	80.65(9.57)	77.62(10.44)	85.00(17.32)	0.380
Baseline ADP(5mM)	76.36(13.56)	79.21(8.54)	86.67(23.09)	0.372
Baseline ADP(10mM)	78.26(12.30)	79.05(8.75)	82.50(20.62)	0.799

Figure 1.Aspirin responder

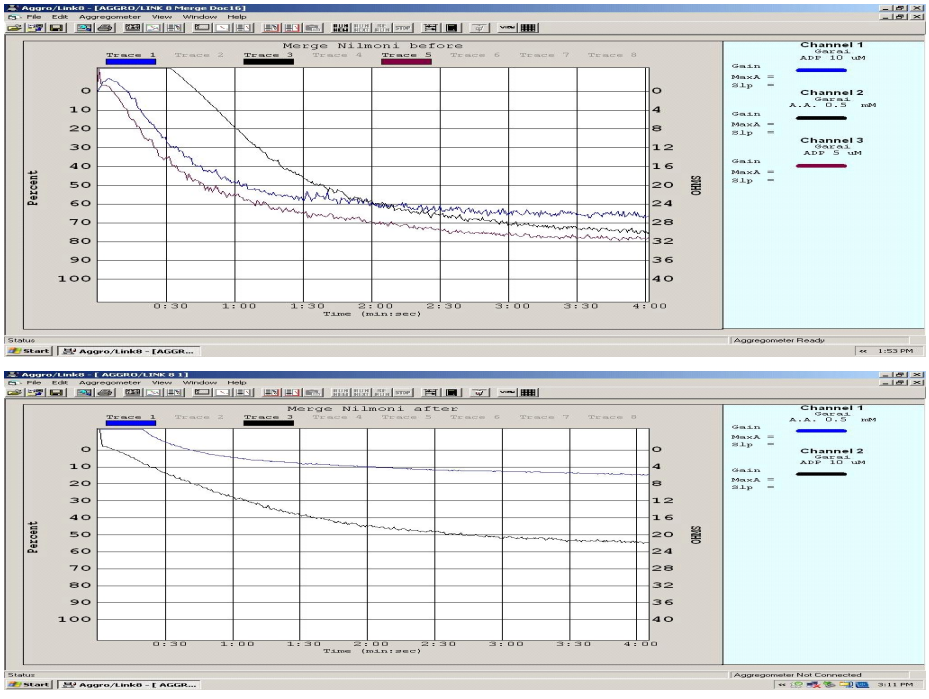
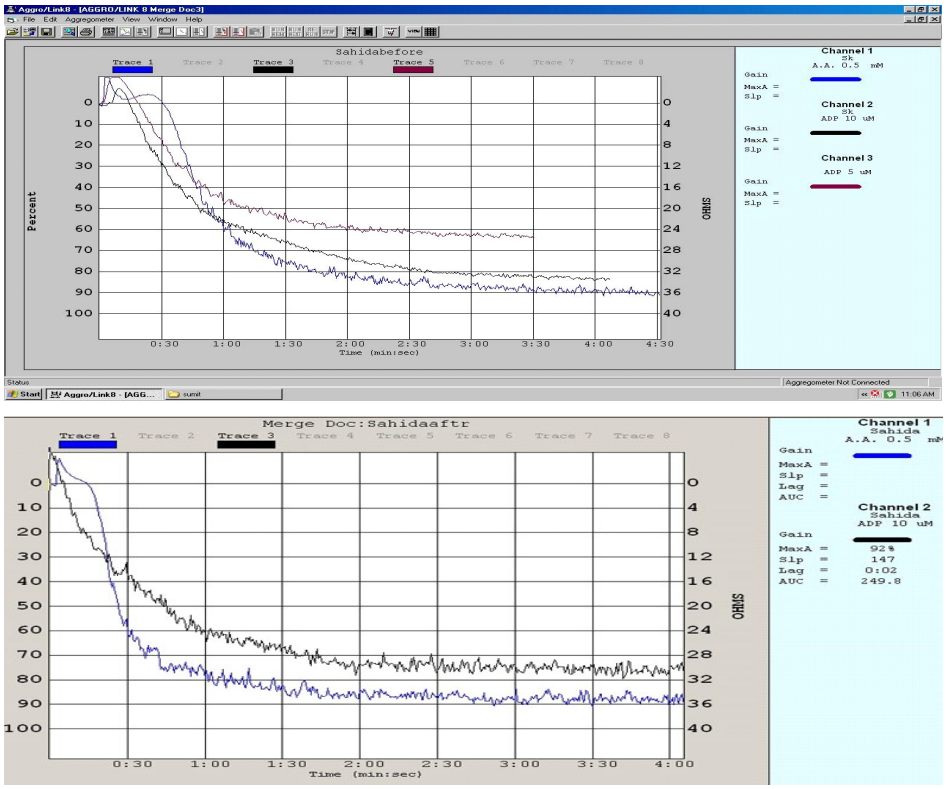


Figure 2.Clopidogrel resistance



Discussion:

High platelet reactivity , along with other factors viz endothelial dysfunction, impaired fibrinolysis, and increased level of coagulation factors contribute to the prothrombotic state in diabetes mellitus. Antiplatelet therapy is an important measure for primary and secondary prevention of atherothrombotic complications in such patients(1,3,4). High platelet reactivity has been observed to persist even with dual antiplatelet therapy in cases of diabetes compared with nondiabetic patients(1,5).

A number of clinical studies have addressed the issue of resistance to antiplatelet therapy. Though the response to Aspirin shows inter and intra individual variability, the unpredictable response can be attributed to clinical, cellular and genetic factors. (11,12). The clinical factors include failure to prescribe, noncompliance, nonabsorbance and drug interaction. A number of clinical studies have correlated aspirin resistance with long term adverse clinical outcomes not only in patients with diabetes but also in individuals with coronary artery disease, ischemic stroke and and peripheral artery disease(13). The prevalence of Aspirin resistance reported by the investigators worldwide varies considerably and these variations are due to differences in the definition of resistance, types of assay used, dose of Aspirin etc (7,9,14,15).

In the present study, twenty three out of 48 patients tested for response to Aspirin were found to be responders (47.9%) while the rest showed inadequate response. The latter group includes both semiresponders and resistant cases. In a study by Sadiq et al on north Indian patients of coronary artery disease, a total of 41.7% inadequate responses (2.1% Aspirin resistances and 39.6% semiresponders) was observed (9). However , this study was done with an Aspirin dose of 150 mg per day, but the methodology was the same as our study. In another study from North India , Aspirin resistance was assessed in patients with ischemic stroke. 150mg

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Aspirin was administered per day, and prevalence of Aspirin resistance was found to be 36%. (16). The authors had found total leucocyte count to be significantly associated with Aspirin resistance. In our study , difference in serum creatinine was statistically significant in the three groups , but it must be mentioned that only four cases of Aspirin resistance were detected in our study . Hence , a larger study is necessary to corroborate our findings.

In a study from eastern India, ninety four patients of Acute coronary syndrome were assessed for Aspirin and Clopidogrel resistance. The authors noted that resistance to both these drugs were comparable and that serum fibrinogen and CRP levels showed strong correlation with antiplatelet resistance. (17)

Hyperglycemia has been considered to have a role in aspirin resistance and may explain why no differences in aspirin resistance has been shown when comparing patients with type-1 and type-2 diabetes mellitus.(18) Furthermore, an interaction between glycation and acetylation has been repeatedly demonstrated. Increased glycation of platelet and coagulation factor proteins may interfere with the acetylation process, thereby contributing to aspirin resistance in the presence of diabetes mellitus. It remains unclear whether improved glycemic control enhances the efficacy of aspirin, or if increased doses of aspirin are beneficial in the presence of poor glycemic control. Currently, there is a lack of evidence supporting specific strategies to overcome aspirin resistance in diabetes mellitus, including the use of a higher dose or the concomitant use of another antiplatelet agent such as clopidogrel.(19) Regarding response to Clopidogrel our findings about resistance to the drug was similar to those by Guha (20), Kumar (10) and Kar et al (21). Several studies have focussed on how to overcome Clopidogrel nonresponsiveness. Current guidelines provide only a weak recommendation for increasing the maintenance dose of Clopidogrel to 150 mg per day in those patients with Clopidogrel resistance in whom stent thrombosis may be catastrophic or lethal (22). Thus we see there are no reliable clinical predictors for this condition. The diagnosis relies primarily on a laboratory assessment of platelet functions. Further, we need to formulate a policy on aspirin usage and ascertain whether all patients taking aspirin need to be investigated, whether all patients with so-called aspirin resistance be put on clopidogrel and lastly whether there is a serious issue of clopidogrel resistance at hand as well(23,24).

However, our study has certain limitations.. This is a small study of only 52 patients. Aspirin and Clopidogrel resistance have been measured only once after relying on the patients' history. No follow up on the patient has been done and this may have a bearing on the final outcome. Furthermore no investigations at other drug doses, eg Aspirin at 150 mg per day or Clopidogrel at 300 mg has been done. Thus a comprehensive picture of antiplatelet resistance is not obtained.

Conclusion:

Thus we see that the prevalence of anti platelet drug resistance in eastern India is similar to that reported by other studies. Aspirin can be initiated for most patients. Clopidogrel can be used as a second line agent in case of contraindication to Aspirin or adverse effects due to the latter. The minimum dose has been used for both the drugs. Thus the cost of therapy can be lowered, benefitting a huge population.

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