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Full Length Research Paper

Addition of clopidogrel versus cilostazol in acute ischemic stroke patients while on aspirin therapy

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Antiplatelet therapy is the cornerstone for the secondary prevention in acute ischemic stroke. Aspirin was considered first-line therapy for secondary prevention in patients with atherothrombotic stroke. However, several studies actually found that aspirin alone did not significantly regulate platelet activation or aggregation in acute ischemic stroke. Thus, a rational approach, to augment this relatively weak inhibitory effects, is to combine it with an anti-platelet agent with a different mechanism of action. This study compared the efficacy and safety of dual antiplatelet therapy of aspirin and clopidogrel versus aspirin and cilostazol in non-cardioembolic stroke patients who were on current aspirin therapy admission. 60 patients with acute ischemic stroke were randomized to receive either aspirin (162 mg/d) plus clopidogrel (75 mg/d) or aspirin (162 mg/d) plus cilostazol (200 mg/d)for six months. The antiplatelet effect of dual therapy was monitored by measuring P-selectin and PAC-1 expression before and after 7days of therapy. The clinical efficacy was assessed using National Institute of Health Stroke Scale and modified Rankin Scale. The composite clinical endpoint (death, recurrent stroke, hemorrhagic complications, hemorrhagic transformation), was used to assess the safety of the two treatment strategies. Both regimens were similarly effective in significantly reducing P-selectin and PAC-1 expressions in the acute phase of ischemic stroke. Patients on both regimens showed a significant improvement in the neurological and functional outcome during the hospitalization period with no significant difference. A non significant higher incidence of hemorrhagic complications and recurrent stroke was encountered among patients on aspirin plus clopidogrel therapy than those on aspirin plus cilostazol therapy. Dual antiplatelet therapy of either aspirin plus clopidogrel or aspirin plus cilostazol was effective in improving the therapeutic outcomes of patients admitted with acute ischemic stroke while on current aspirin therapy.

Keywords: Clopidogrel, cilostazol, ischemic stroke, platelet, P-selectin, aspirin therapy.

INTRODUCTION

Platelet activation and aggregation are critically involved in the pathophysiology of atherosclerosis and thrombosis (1) and play a great role in the pathophysiology of ischemic stroke. Recently, a number of studies have shown that platelets are activated in the acute phase of ischemic stroke (2). Upon activation, platelets secrete P-selectin which mediates platelet aggregation and platelet-leukocyte interactions. Both are important mechanisms in the development of arterial thrombosis in ischemic stroke (3). Procaspase activating compound-1 (PAC-1) is a monoclonal antibody that recognizes an epitope on the GPIIb-IIIa complex located near the platelet fibrinogen

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receptor that is expressed only upon platelet activation (4). Both PAC-1 and P-selectin adhesion proteins are sensitive surface markers of platelet activation, which can be evaluated in samples of whole blood by flow cytometry (5).

Antiplatelet therapy has become the cornerstone for primary as well as for secondary prevention of stroke (6). Antiplatelet agents provide not only a preventive role in acute thrombosis, but they can also inhibit the platelet contribution to atherosclerotic lesion progression and modulate the inflammatory process (7).

Aspirin was considered first-line therapy for secondary prevention in patients with atherothrombotic stroke because of its good safety profile and low cost(8). However, several studies actually found that aspirin alone did not significantly regulate platelet activation or aggregation in acute ischemic stroke (9-10). Moreover, other large clinical studies have found that 30–40% of patients who had a stroke were taking aspirin at the time of their event (11-12)**.** It was also reported that about 10–20% of aspirin-treated patients experience recurrent ischemic events within 5 years of administration (13). Moreover, patients who have ischemic events while taking aspirin may have worse outcomes than those not receiving aspirin (14). Thus, a rational approach, to augment this relatively weak inhibitory effects, is to combine it with an anti-platelet agent with a different mechanism of action (15). Examples include ADP receptor blockers (clopidogrel or ticlopidine) or phosphodiesterase inhibitors (dipyridamole or cilostazol) (16).

Hence the aim of this work was to clinically try clopidogrel versus cilostazol as an add-on-therapy to asprin in such a context and assess their clinical efficacy and safety.

PATIENTS AND METHODS

Study population

From September, 2009 to November, 2010, consecutive patients with acute ischemic stroke admitted to Ain Shams Specialized Hospital, within 24 hours of onset of symptoms prior to randomization, were evaluated. A stroke diagnosis was established based on clinical presentation, neurologic examination, and results of a brain computed tomography (CT) and magnetic resonance imaging (MRI) with magnetic resonance angiography (MRA). Patients aged 40 to 75 years, who were on previous aspirin therapy of 75-150 mg/day dose for at least 2 weeks before enrollment, were included if they suffered acute non-cardioembolic ischemic stroke. Exclusion criteria were cardioembolic stroke that requires treatment with anticoagulant therapy, hemorrhagic stroke or history of intracranial hemorrhage, major surgery or major trauma within the preceding three months, thrombolytic therapy (tissue-type plasminogen activator) within the preceding 24 hours, known malignancy or hematologic disorders that may affect platelet count or function e.g. thrombocytopenia (platelet count < 100,000/mm3), Medical contraindication to the use of aspirin (peptic ulcer disease or hypersensitivity) or clopidogrel (bleeding diathesis) or cilostazol (congestive heart failure of any severity), concurrent use of other antiplatelet drugs or non steroidal anti-inflammatory drugs (e.g. ibuprofen), severe renal impairment (sr.creatinine >2 mg%) and moderate to severe liver impairment (ALT and/or AST > 2 times the upper normal limit).

Ethical aspects

This clinical study was performed in compliance with Good Clinical Practice and the principles of the Declaration of Helsinki (2000) and subsequent revisions. Ethical approval for the study was granted by the Ethical committee of Ain Shams University. Informed consents were taken from all patients or their caregivers.

Study design

This work was a prospective, randomized, single-centered, parallel-group study. A total of sixty (60) patients with acute ischemic stroke were evaluated during the study and were randomly assigned to enter the two treatment groups using table of block randomization. The first group comprised 30 patients that received dual antiplatelet therapy of aspirin (162 mg/day) plus clopidogrel (75 mg/day). The second group comprised 30 patients that received dual antiplatelet therapy of aspirin (162 mg/day) plus cilostazol (200 mg/day). The trial therapy was continued for 6 months in both groups.

Measurements

All patients underwent detailed history taking, complete clinical examination with special emphasis on the neurological signs of ischemic stroke using National Institute of Health Stroke Scale (NIHSS) (17) at the time of admission and on discharge to assess the neurological outcome of patients, and the symptoms of physical disability using modified Rankin Scale (mRS) (18) at admission, after 2 weeks, 3 months and 6 months follow-up period to assess the functional outcome of patients. Laboratory investigations including serum electrolyte levels, complete blood count (platelet count), blood glucose, liver and kidney functions. ECG, Echocardiography, brain CT and MRI with MRA were also carried out. The MRI with MRA findings were classified according to *the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria*(19) into: large-vessel/small vessel ischemic stroke, according to site of infarction into: cortical, cortical/subcortical, subcortical (including lacunar infarcts and large infarcts), and according to the number of infarctions bilaterally into:

single/multiple infarcts.
Large vessel ischemic stroke was diagnosed by both MRA and extracranial carotid duplex sonography showing a stenosis of the brain supplying arteries with a 50% diameter reduction and a typical morphology for atherosclerotic lesions. Small vessel ischemic stroke was diagnosed if neuroimaging showed ischemic lesions smaller than 1.5 cm with clinical symptoms consistent with typical lacunar syndromes (i.e. pure motor stroke, pure sensory stroke, sensorimotor stroke, dysarthria-clumsy hand syndrome, and ataxic hemiparesis).
The platelet activation markers (P-selectin and PAC-1) using flow cytometry technique were also assessed at baseline according to methodology described in previous study (20). After 7 days of administration of dual antiplatelet therapy, P-selectin and PAC-1 expression were reassessed to monitor the antiplatelet efficacy of the dual therapy. Any adverse clinical outcome during hospital stay and up to the end-time of the trial therapy was assessed including: Major hemorrhage (any hemorrhage that is life threatening or require blood transfusion; either intracranial from hemorrhagic transformation of the infarct or extracranial), minor hemorrhage (bleeding at sites of injection, gum bleeding, epistaxis, ecchymosis hematuria or GIT bleeding), death of any cause, progressing stroke symptoms (deficit that continue to worsen in spite of antiplatelet therapy) and recurrent stroke (defined as any new fatal or non-fatal event, ischemic or hemorrhagic, subsequent to the initial one, with a new neurological deficit or an increased impairment of the previous deficit, persisting beyond 24 hours). A composite clinical end point of death, recurrent stroke, transient ischemic attack, hemorrhagic complications or hemorrhagic transformation and deterioration in the MRS were used to assess the clinical efficacy and safety of the two antiplatelet treatment strategies

Statistical analysis

Comparisons between groups were done using student t-test for parametric data and Mann-Whitney U test for non-parametric data. Chi-square test was used to compare the two groups as regards the categorical data. Repeated measures of ANOVA were used to compare MRS at 4 different time points (on admission, after 2 weeks, after 3 and 6 months). Paired t-test was used to compare means on the same group over time in a before-after situation to monitor the efficacy of therapy.
Multi-variable logistic stepwise regression analysis was used to determine independent predictors for adverse clinical event. $P < 0.05$ was considered statistically significant. All statistical calculations were performed using the SPSS statistics (V. 19, IBM Corp., USA, 2010).

RESULTS

Baseline clinical characteristics

There was no significant difference between the two groups as regards the baseline clinical characteristics (Table 1).

Laboratory and Neuroimaging findings

There was no significant difference in the laboratory and neuroimaging findings between both groups (Table 2).

Neurological and Functional outcome

There was a non significant difference between both groups regarding NIHSS on admission and on discharge ($p=0.415$ and $p= 0.141$ respectively). However, there was a highly significant decrease in the median values of NIHSS in both aspirin plus clopidogrel and aspirin plus cilostazol groups from admission to discharge ($p<0.001$ and $p<0.001$ respectively) (Figure 1).
The percentage of patients with improved neurological outcome was not significantly different between both groups (90% versus 76.6%, $p=0.299$) (Figure 2).
In both aspirin plus clopidogrel and aspirin plus cilostazol groups, there was a highly significant decrease in the median values of mRS after 2 weeks ($p=0.01$ and $p<0.001$ respectively), after 3 months ($p=0.001$ and $p<0.001$ respectively) and after 6 months ($p=0.001$ and $p<0.001$ respectively) as compared to baseline. However, the change was more among aspirin plus cilostazol group but the difference was non-significant ($p>0.05$) (Figure 3).
At the end of 6 months follow-up, the percentage of patients with improved functional outcome was significantly higher in aspirin plus cilostazol group than in aspirin plus clopidogrel group (83.3% versus 56.7% respectively). Consequently, the percentage of patients with deteriorated functional outcome among the aspirin plus clopidogrel group was significantly higher than aspirin plus cilostazol group (16.6% versus 0% respectively; $p= 0.036$) (Figure 4).

Adverse clinical events

There was a non significant higher incidence of hemorrhagic complications (mainly major extracranial and minor bleeding) ($p= 0.064$) and recurrent stroke ($p= 0.3$) among patients on aspirin plus clopidogrel therapy than those on aspirin plus cilostazol therapy. In general, there was a non significant difference in the incidence of the composite end point of death, recurrent stroke and hemorrhagic complications between both groups ($p= 0.095$). However, these results were clinically significant with lower events rate and bleeding risks among the patients in the aspirin plus cilostazol group (Table 3).

Platelet activation markers

On admission, there was no significant difference in the median values of P-selectin and PAC-1 between both groups ($p = 0.606$ and $p = 0.433$ respectively). After 7 days, there was a highly significant decrease in P-selecting

Table 1. The Baseline clinical characteristics and Medication history of aspirin plus clopidogrel and aspirin plus cilostazol groups.

Characteristics		***Aspirin +Clopidogrel (no.=30)***	***Aspirin + Cilostazol no.=30)***	***p-value***
Age (years) (Mean ± S.D.)		*62.73 ±11.62*	*60.16±8.09*	*0.483*
SEX	*Male*	*17 (56.7%)*	*16 (53.3%)*	*0.795*
	Female	*13 (43.3%)*	*14 (46.7%)*	
Systolic BP (Mean ± S.D.)		*157.42 ±24.3*	*153.6±27.3*	*0.568*
Diastolic BP (Mean ± S.D.)		*96.9 ±18.95*	*93.5 ± 14.1*	*0.41*
Heart rate (Mean ± S.D.)		*91.69 ±14.36*	*100.6 ±23.1*	*0.077*
Hypertension		*24 (80%)*	*27 (90%)*	*0.474*
Diabetes mellitus		*18 (60 %)*	*21 (70%)*	*0.535*
Hyperlipidemia		*17 (56.7%)*	*16 (53.3%)*	*0.98*
Ischemic heart disease		*17 (56.7%)*	*18 (60%)*	*0.845*
Smoking		*20 (66.6%)*	*17 (56.6%)*	*0.632*
Family history		*11 (36.6%)*	*9 (30%)*	*0.959*
Previous stroke		*9 (30%)*	*11 (36.6%)*	*0.424*
Previous TIA		*12 (40%)*	*8 (26.6%)*	*0.364*
Medication history	*βeta-Blockers*	*8 (26.7%)*	*11 (36.7%)*	*0.1*
	ACEIs	*17 (56.6%)*	*12 (40%)*	*0.13*
	Ca-Blockers	*4 (13.3%)**1***	*7 (23.3%)*	*0.21*
	Hypoglycemics	*18 (60%)*	*21 (70%)*	*0.59*

BP= blood pressure, TIA= transient ischemic attack, ACEIs = angiotensin converting enzyme inhibitors, Ca = calcium .
p-value indicates the difference between the groups with non-significant difference at p > 0.05.

Table 2. The Baseline laboratory findings and the Neuroimaging findings (MRI with MRA) of both aspirin plus clopidogrel and aspirin plus cilostazol groups.

Parameter			***Aspirin +Clopidogrel (no.=30)***	***Aspirin + Cilostazolno.=30)***	***p-value***
Serum Creatinine (mg/dL) (Median (IQR))			*1 (0.8-1.32)*	*0.8 (0.6-1.1)*	*0.07*
Blood Urea Nitrogen (mg/dL) (Mean ± SD)			*19.24 ± 9.64*	*18.2 ± 6*	*0.63*
Alanine Transaminase (U/L) (Median (IQR))			*22.5 (14.7- 31.5)*	*22 (16.7- 33)*	*0.65*
Aspartate Transaminase (U/L) (Median (IQR))			*25 (21- 30)*	*23.5 (20.7-27.7)*	*0.58*
Creatine Kinase -MB (U/L) (Median (IQR))			*20 (16.75- 30.5)*	*17 (14-.7-22.2)*	*0.06*
Platelet count (/mm^3) (Median (IQR))			*271 (226.5- 304)*	*225.5 (186.2-290.5)*	*0.11*
Hemoglobin (gm %) (Mean ± SD)			*13.37 ± 2.03*	*13.07 ± 2.01*	*0.55*
Glycated Hemoglobin (%)(Mean ± SD)			*7.05 ± 2.27*	*7.19 ± 2.16*	*0.3*
Total cholesterol (mg/dL) (Mean ± SD)			*204 ±56.77*	*188 ± 43.5*	*0.213*
Low Density Lipoprotein-C (mg/dL) Median (IQR))(			*135 (90- 156)*	*125.5 (106.2- 141.2)*	*0.42*
High Density Lipoprotein-C (mg/dL) (Mean ± SD)			*40.03 ± 12.83*	*36.36 ± 8.67*	*0.186*
Triglyceride (mg/dL) (Median (IQR))			*147 (107-201)*	*120.5 (96.2-208.5)*	*0.47*
Fasting Blood Glucose (mg/dL) (Median (IQR))			*130 (95- 236)*	*172.5 (106.7-223)*	*0.44*
MRI with MRA findings	*Size of infarction*	*Small-vessel disease*	*18 (60%)*	*19 (66.7%)*	*0.326*
		Large-vessel disease	*12 (40%)*	*11 (33.3%)*	
	Site of infarction	*Cortical*	*11 (36.6%)*	*9 (30%)*	*0.28*
		Cortical/Subcortical	*7 (23.4%)*	*6 (20%)*	
		Subcortical	*12 (40%)*	*15 (50%)*	
	Number of infarctions	*Single (no.%)*	*17 (56.7%)*	*18 (60%)*	*0.134*
		Multiple (no.%)	*13 (43.3%)*	*12 (40%)*	

MRI = Magnetic Resonance Imaging, MRA = Magnetic Resonance Angiography, IQR= interquartile range
p-value indicates the difference between the groups with non significant difference at p > 0.05.

and PAC-1 expression compared to baseline in both aspirin plus clopidogrel and aspirin plus cilostazol groups(P-selectin; p=0.001 & p <0.001 respectively, PAC-1; p=0.001 &p <0.001 respectively), however the change was

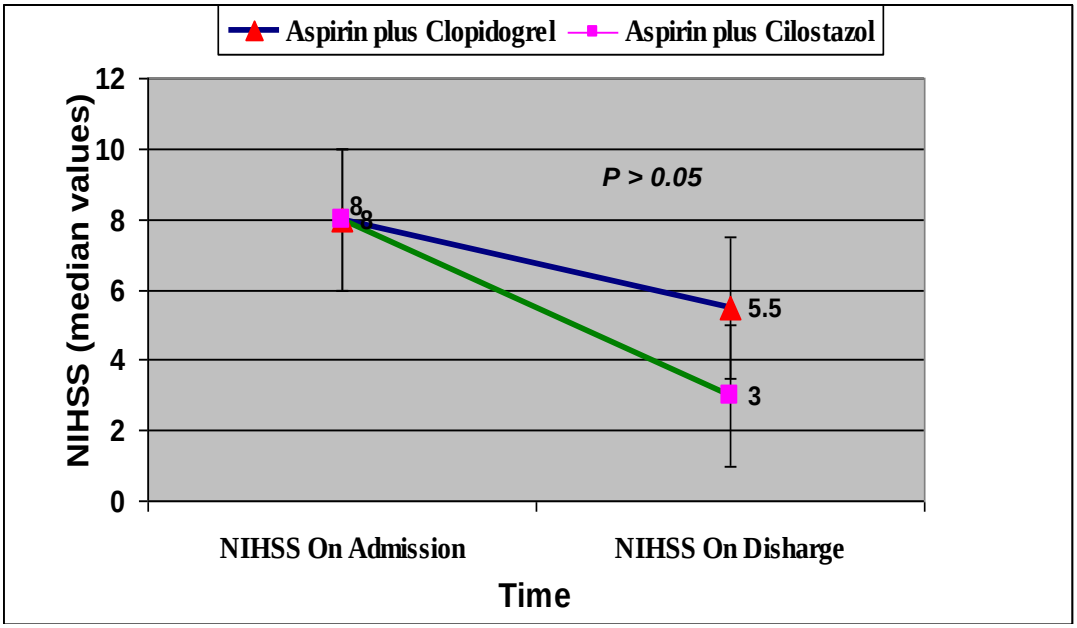

Figure 1. The changes in NIHSS in both Aspirin plus Clopidogrel and Aspirin plus Cilostazol groups.

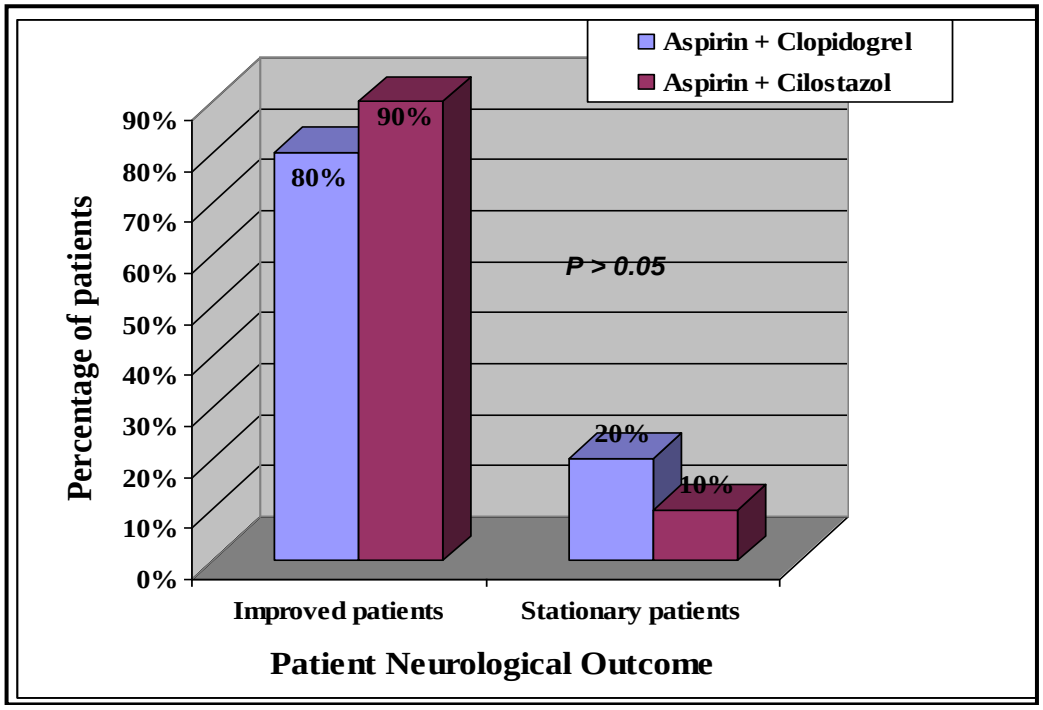

Figure 2. The Effect of Aspirin plus Clopidogrel and Aspirin plus Cilostazol therapies on the Neurological outcome of patients on discharge.

more among aspirin plus cilostazol group (Figures 5 & 6). No significant difference was noted between both groups regarding the percentage inhibition of P-selectin and PAC-1 expression (% positive cells) over 7 days (p= 0.156 andp= 0.383 respectively) (Table 4).

Predictors of an adverse clinical event

Statistical analysis was done between poor outcome (11 patients) (those who experienced an adverse clinical event of hemorrhage or recurrent stroke) and good

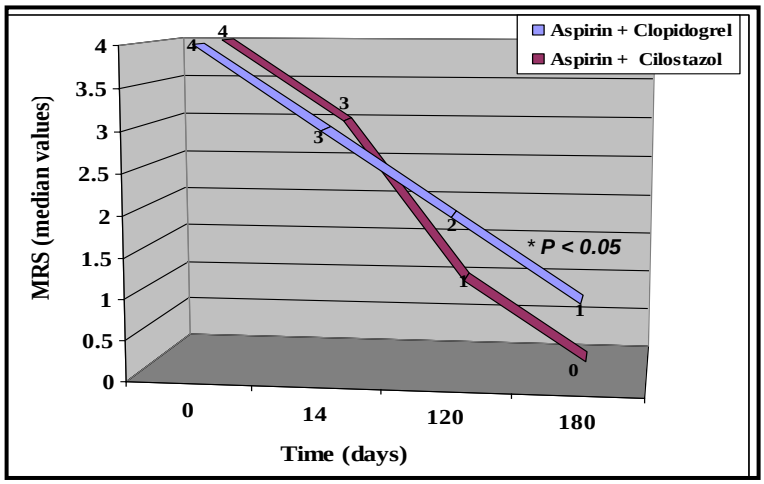

Figure (3): The changes in MRS at different time periods in both Aspirin plus Clopidogrel and Aspirin plus Cilostazol groups.

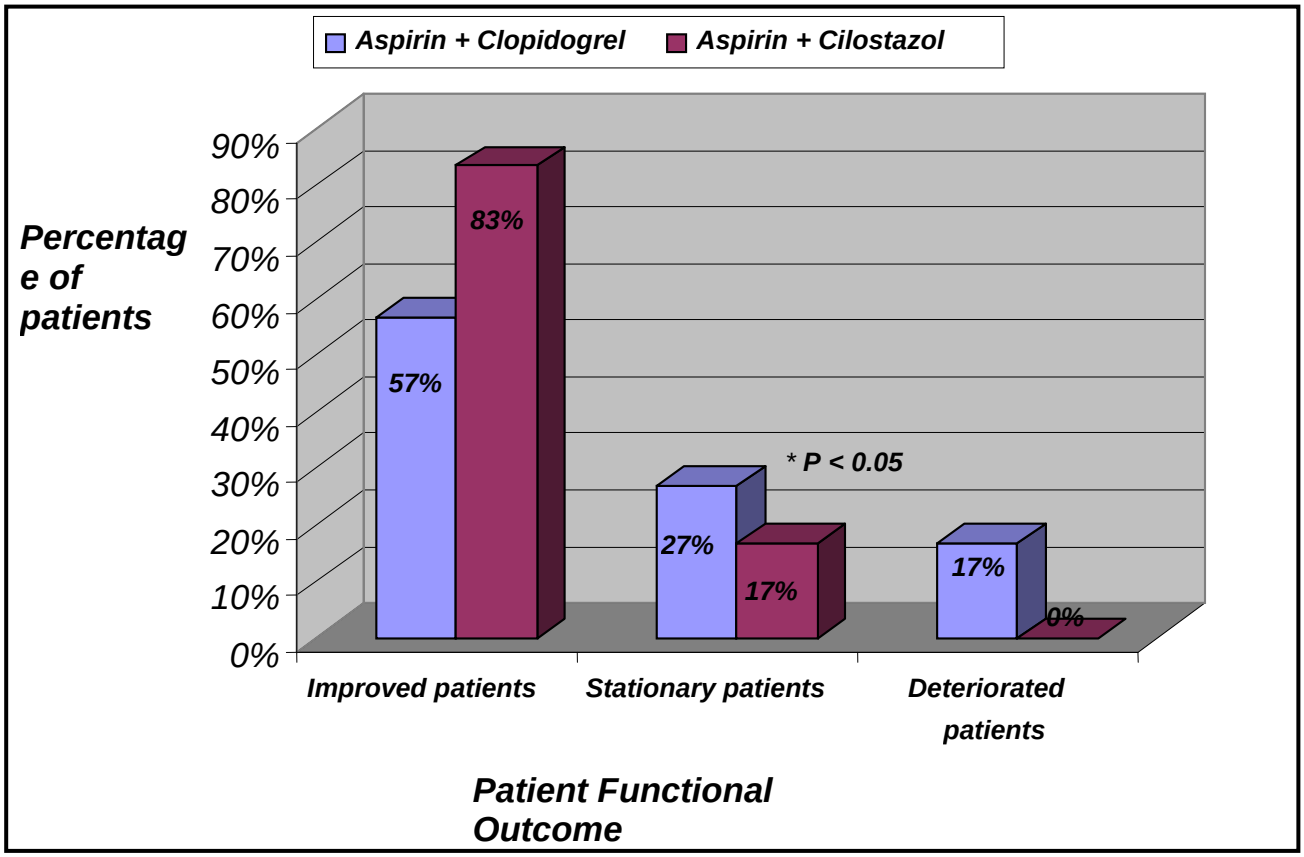

Figure 4. The Effect of Aspirin plus Clopidogrel and Aspirin plus Cilostazol therapies on the Functional outcome of patients after 6 months.

outcome (49 patients) (those who had not experienced an adverse clinical event of hemorrhage or recurrent stroke). There was a highly significant difference between poor outcome and good outcome patients as regards neuroimaging findings and percentage inhibition of P-selectin and PAC-1 (Tables 5 & 6). Multivariate analysis showed that P-selectin was the independent predictor for the adverse clinical event in acute ischemic stroke

Table 3. The Adverse clinical outcome and Incidence of composite clinical endpoint in both groups during 6 months follow up period.

	Aspirin +Clopidogrel (no.=30)	***Aspirin + Cilostazol no.=30)(***	***p-value***
Adverse clinical outcome			
1 - Hemorrhage			
(a) Major Hemorrhage[a]	*3 (10%)*	*0 (0%)*	
-Intracranial	*0 (0%)*	*0 (0%)*	*0.064*
-Extracranial	*3(10%)*	*0 (0%)*	
(b) Minor (only extracranial)	*2 (6.6%)*	*2 (6.6%)*	
2- Recurrent stroke	*3 (10%)*	*1 (3.3%)*	*0.3*
Incidence of composite end point			
Composite end point	*8 (26.6)*	*3 (10)*	*0.095*
No Composite end point	*22 (73.4)*	*27 (90)*	

(a) Treatment was stopped in these patients within 48 hours.
P-value indicates a non significant difference between both groups at p-value >0.05.

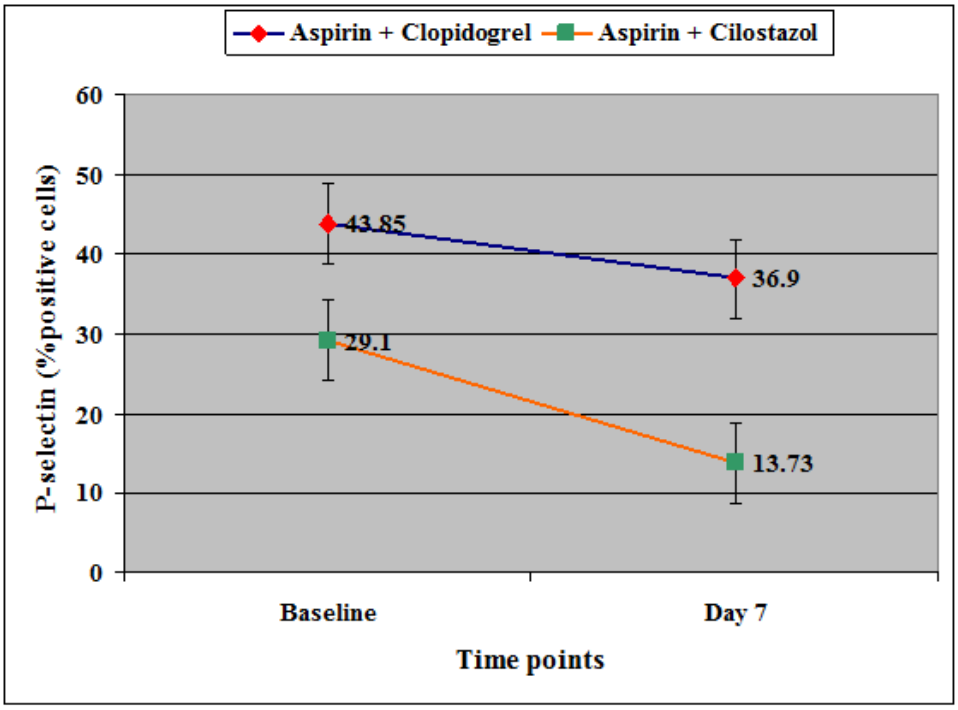

Figure 5. The Sequential changes of P-selectin in both Aspirin plus Clopidogrel and Aspirin plus Cilostazol group.

patients (odds ratio = 1.275 and 95% confidence interval of 1.06 to 1.29(p= 0.005).

DISCUSSION

The present study demonstrated that dual antiplatelet therapy of either aspirin plus clopidogrel or aspirin plus cilostazol was similarly effective in improving the neurological and functional outcomes of patients admitted with acute ischemic stroke while on previous aspirin therapy. However the rate of improvement was higher among patients on aspirin plus cilostazol therapy. In addition, aspirin plus cilostazol regimen was associated with lower events rate and lower bleeding risks.

The clinical efficacy of these dual antiplatelet regimens might be explained by the significant inhibition of P-selectin and PAC-1 expression on activated platelets after 7 days of administration of both dual antiplatelet regimens compared to baseline in the acute phase. However, the

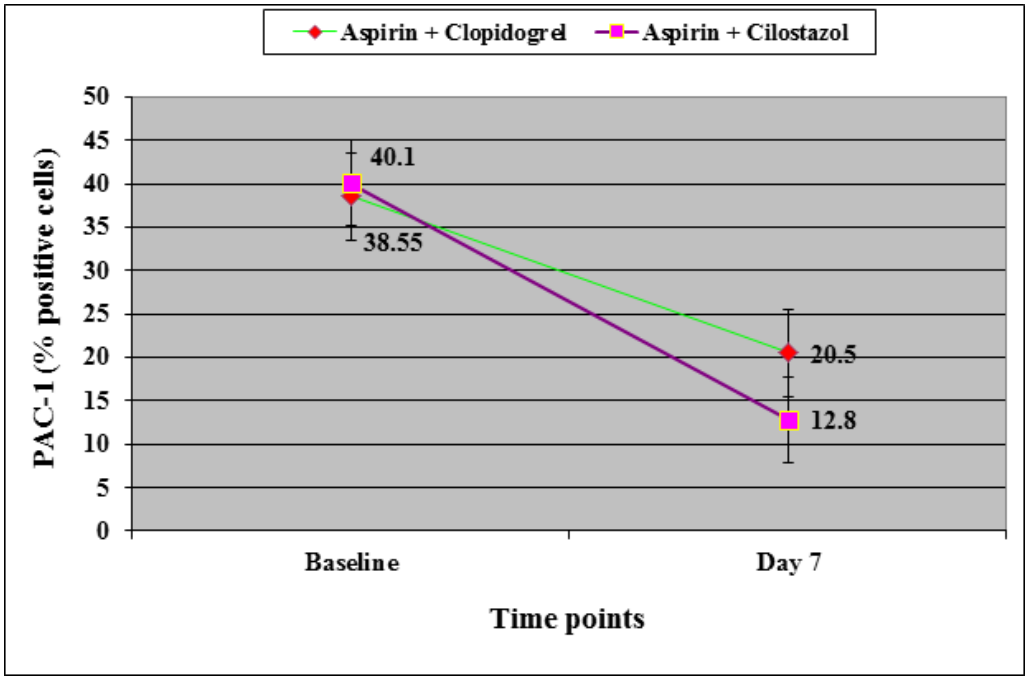

Figure 6. The Sequential changes of PAC-1 expression in both Aspirin plus Clopidogrel and Aspirin plus Cilostazol groups

Table 4. The percentage inhibition of P-selectin and PAC-1 expression among aspirin plus clopidogrel and aspirin plus cilostazol groups.

Parameter	*Aspirin + Clopidogrel (no.=30)*	*Aspirin + Cilostazol no.=30)(*	*p-value*
% inhibition P-selectin Median (IQR)	*54.3 (23.4-74.8)*	*63.8 (35.2-83.2)*	*0.156*
% inhibition PAC-1 Median (IQR)	*35.7 (18.4-64.2)*	*46.3 (25.2-73.2)*	*0.383*

IQR =Interquartile range
P-value indicates no significant difference between groups at p-value>0.05.

Table 5. The Neuroimaging findings of Good outcome and Poor outcome groups.

Neuroimaging findings		*Good outcome no. = 49*	*Poor outcomeno. = 11*	*p-value*
According to type of infarctions	*Small-vessel disease*	*35 (71.4%)*	*2 (18.2%)*	*0.001**
	Large-vessel disease	*14 (28.6%)*	*9 (81.8%)*	
According to site of infarctions	*Cortical*	*12 (24.5%)*	*8 (72.7%)*	*0.005**
	Cortical or Subcortical	*11 (22.5%)*	*2 (18.2%)*	
	Subcortical	*26 (53%)*	*1 (9.1%)*	
According to number of infarctions	*Single (no.%)*	*33 (67.3%)*	*2 (18.2%)*	*0.001**
	Multiple (no.%)	*16 (32.7%)*	*9 (81.8%)*	

(*) p-value indicates the difference between both groups with a highly significant difference at p-value <0.01.

change was more among patients on aspirin plus cilostazol regimen.

Moreover, there was a significant correlation between the elevation in P-selectin and PAC-1 expressions and poor outcome in ischemic stroke patients. The present study demonstrated that P-selectin was the independent predictor for poor outcome in ischemic stroke patients such that increased P-selectin expression could predict the

Table 6. The percentage inhibition of P-selectin and PAC-1 in Good outcome and Poor outcome groups.

Parameter	*Good Outcome no. = 49*	*Poor Outcome no. = 11*	*p-value*
% inhibition P-selectin Median (IQR)	*57.23 (21.3- 73.8)*	*10 (5.2 - 18.5)*	*<0.001**
% inhibition PAC-1 Median (IQR)	*59.54 (20.2- 79.2)*	*19.5(10.21-22.3)*	*<0.001**

(*) p-value indicates the difference between both groups with a highly significant difference at p-value <0.01.

exposure of these patients to recurrent attacks of stroke by 27.5%.

The rational use of combined antiplatelet therapy in the current study was supported by previous reports. They stated that the effects of aspirin might be too weak to curb platelet activation in acute ischemic stroke, and patients who have ischemic events while taking aspirin may have worse outcomes than those not receiving aspirin (9, 14). In addition, others reported that the blocking of only one of several platelet activation pathways might not regulate platelet aggregation in thrombotic stroke (21).

Actually regarding the use of dual aspirin and clopidogrel regimen in the setting of acute ischemic stroke, there was much debate. Several studies have shown beneficial effects of dual aspirin clopidogrel therapy in some stroke patients. It was reported that combining antiplatelet regimens of aspirin and clopidogrel with different mechanisms of action may further improve long-term clinical outcomes especially in patients who experienced a clinical event while on aspirin therapy (15). Similarly, the PLUTO-stroke trial found that treatment with clopidogrel plus aspirin for 1 month provides significantly greater inhibition of platelet activity than aspirin alone in patients after recent ischemic stroke in the frame of the small randomized trial. There were no deaths, hospitalizations, or serious adverse events (22). Recently, the most important finding of the SAMMPRIS trial was the impressive reduction of the rate of stroke with the use of aggressive medical therapy of aspirin and clopidogrel for 90 days as compared with the use of percutaneous transluminal angioplasty and stent (PTAS) (5.8% versus 14.7%) among patients with non-disabling stroke within 30 days before enrollment. The rates of any major hemorrhage were also significantly higher in the PTAS group than in the medical-management group (23).

On the other hand, Diener et al. (24) reported that the clopidogrel-aspirin combination did not significantly decreased the rate of ischemic events (MI or stroke) after a follow up period of 18 months among patients who had TIA or recent stroke within the prior three months, even more the risk of hemorrhage was increased in the clopidogrel-aspirin group.

On the other side, several previous studies had reported the efficacy of cilostazol therapy as compared to aspirin therapy in significantly decreasing the incidence of recurrent stroke without increasing occurrence of cerebral haemorrhage (25-26).

In concordance with the present study, Lee et al. (27) reported that addition of cilostazol to ischemic stroke patients with aspirin treatment failure has reduced the incidence of aspirin resistance from 12.8% to 8.8% in cilostazol group, without increasing the bleeding risks.

The reduced risk of stroke with cilostazol in such trials that is not associated with increased bleeding risks can be ascribed not only to its antiplatelet properties but also to the improvement of vascular endothelial function and anti-inflammatory effects, which may be prophylactic against bleeding (28). In addition, its vasodilatation properties caused by increased production of nitric oxide, an endogenous vasodilating factor, and reduction of intracellular calcium ion concentrations (29).

In contrast, aspirin and clopidogrel, which act exclusively on platelets, have limited effects on cerebrovascular events with 20% to 25% reduction (30) and can increase risk of serious bleeding even in monotherapy and especially in combination (31).

In concordance with the present study, Cha et al. (32) declared that potent synergy effects of clopidogrel and aspirin regarding the inhibition of platelet hyperactivation led to a decrease in P-selectin expression on platelets and consequently halt down the progression of atherthrombosis and neuronal injury in acute ischemic stroke. Furthermore, Labarthe et al. (33) demonstrated that aspirin alone had no detectable effect on P-selectin expression nor on PAC-1 binding while upon addition of clopidogrel, P-selectin expression was reduced by 66% and PAC-1 binding by 55%.

The Plavix Use for Treatment stroke trial showed little changes in P-selectin expression on activated platelets in ischemic stroke when using the usual doses of aspirin alone or aspirin plus clopidogrel but significant reduction in PAC-1 expression in the aspirin plus clopidogrel (22). This supports a previous crossover study that the combination of clopidogrel and aspirin provides more potent inhibition of platelet function than monotherapy with each agent (34).

Tsai et al. (35) reported that the exact inhibitory mechanism of this combination regimen for P-selectin or PAC-1 on platelets is unknown, however the synergy between clopidogrel and aspirin may be secondary to the different platelet activation pathways inhibited by these antiplatelet drugs suggested that simultaneous antagonism of thromboxane A2 by aspirin and adenosine diphosphate by clopidogrel resulted not only in inhibition of arachidonic

acid-and ADP-mediated platelet activation but also in a reduction of collagen- and thrombin-induced platelet activation. Therefore, we suggest that the concurrent blockade of multiple activation pathways by both clopidogrel and aspirin can effectively down regulate the surface expression of P-selectin and GP IIb/IIIa expression on activated platelets.

On the other side, several studies have demonstrated the effect of Cilostazol on p-selectin expression and GPIIb/IIIa activation. It was reported that cilostazol can reduce the expressions of p-selectin and activated GPIIb/IIIa by adenosine diphosphate (ADP) stimulation (36) and the release of P-selectin from platelets by ADP or collagen stimulation (37).

The efficacy of cilostazol on inhibition of p-selectin expression and GPIIb/IIIa activation could be explained according to Keh et al. (38) and Fullard (39) by that Glycoprotein IIb-IIIa expression and P-selectin release are strictly regulated by activating signals from 5¢-adenosine diphosphate, thrombin and thromboxane A2 (TXA2) receptors, and inhibitory signals from nitric oxide (NO). Cilostazol, as an inhibitor of phosphodiesterase III, increases the level of cyclic adenosine monophosphate (cAMP) by inhibition of the conversion of cAMP to 5¢-AMP and eventually potentiates the glycoprotein IIb-IIIa inhibitory signals (40)and activates the protein kinase enzyme which in turn induces a decrease in the intracellular calcium, thus suppressing the platelet granule release (41). The effect of cilostazol was also potentiated by improving the endothelial protective function (42). Therefore, cilostazol and aspirin combination would be expected to synergistically inhibit platelet aggregation via quite different pathways

In agreement with the results of the present study, Nomura et al. (43) reported that the combination of aspirin and cilostazol significantly regulate PAC-1 expression on activated platelets in chronic atherothrombosis as compared to aspirin alone. Furthermore, Lee et al. (20) found that aspirin plus cilostazol significantly decreased the expression of PAC-1 on activated platelets after 7 days compared to the baseline in acute ischemic stroke, whereas aspirin alone had no effect on PAC-1 expression. However, they found that P-selectin expression on activated platelets were not changed by combined aspirin and cilostazol in acute ischemic stroke. The discrepancy from the results of the current study might be attributed to the retrospective design of the study, that led to a non homogeneous distribution in the study population which in turn may have affected the results.

Cha et al. (9)had showed that the surface expressions of P-selectin on platelets were correlated well with the clinical severity and significantly increased in patients with clinical deterioration (as measured by NIHSS) at 72 hr and 7 days after ischemic insult, suggesting that P-selectin had important role in the progression of ischemic stroke during 7 days. Similarly, Yip et al. (44)found that high P-selectin expression (cut-off value > 3.16%) is strongly associated with adverse clinical outcomes after acute ischemic stroke. On the other hand, Tsai et al.(35) found that CD40 ligand was the independent predictor of poor outcome but there was a trend of higher P-selectin expression on presentation in stroke patients with poor outcome, even though the difference was not significant. The discrepancy between this study and the present one may be attributed to different methodologies (e.g. stroke subtypes of patients on enrollment or heterogeneous anti-thrombotic agents), follow-up periods (one month vs. six months), and statistical methods.

Finally, we conclude that dual antiplatelet therapy of with either clopidogrel or cilostazol improved the clinical outcomes of patients with acute ischemic stroke on current aspirin. This small clinical trial demonstrated the novel comparison between these two remedies in addition to aspirin. Efficacy and safety of both medicines were documented with a predilection to cilostazol. Further large multi-center randomized trials are needed to confirm our results.

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