Kathmandu University Medical Journal (2005) Vol. 3, No. 1, Issue 9, 11-16

Original Article

Platelet aggregometric study on whole blood of patients with ischaemic heart disease

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Abstract
Platelet aggregation is an important in vitro test to assess platelet aggregation response in IHD. The present prospective case control study was undertaken to evaluate the platelet aggregation response in IHD and the effects of aspirin therapy on it. Platelet aggregation was conducted on whole blood by the Chrono- Log whole blood Aggrometer model 540-VS. Various agonists used for platelet aggregation were collagen, ADP, Epinephrine and Thrombin. High platelet aggregation was observed in-patients of IHD as compared to controls by few or all of the reagents used. Platelet aggregation was high in both MI and angina as compared to control cases. However, cases of MI showed higher response than those of angina. Aspirin intake was associated with a decrease in platelet aggregation in patients of IHD. The platelet aggregation response was higher in PRP as compared to whole blood with similar concentration of reagents, however whole blood was equally effective as PRP in detecting hyper-responsive platelets in - patients of IHD.

Key words: Platelet aggregation, Ischaemic heart disease, chrono-log Aggrometer, platelet function.

Ischemic heart disease (IHD) is the name given to a group of closely related disorders arising due to ischemia and imbalance between the demand and supply of heart for oxygenated blood.[1] Platelets play an important role in thrombus formation. By adhering to areas of vascular endothelial damage they liberate a few mitogenic factors, which contribute to the early atherosclerotic lesion.[2] Studies have shown that platelets in patients of IHD are hyperactive and show hyperaggregability with various agonists like adenosine diphosphate (ADP), epinephrine, thrombin and collagen. It is this hyperresponsiveness which is responsible for the thrombotic events leading to IHD. Platelet aggregation is an important and useful in-vitro test to assess platelet function, which can be tested by adding aggregating agents to platelet rich plasma (PRP) or whole blood. This study was undertaken to evaluate platelet aggregation in patients with IHD using chrono-log Aggrometer model 540-VS adding aggregating factors like ADP, collagen, thrombin and epinephrine and to compare the results after therapy with antiplatelet agent, aspirin.

An attempt was also made to compare platelet aggregation in whole blood and PRP.

Material and methods

A total of 35 patients suffering from IHD, including cases with angina, myocardial infarction (MI) & chronic ischaemic heart disease (CIHD) were studied for their platelet aggregation response using various reagents. The patients were selected from casualty, medicine intensive care unit and cardiology outpatient department of Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry. Patients belonging to this study group comprised of freshly diagnosed cases of IHD as well as old cases of IHD who hade discontinued treatment for a period of at least two weeks.

At the same time 20 control subjects were studied who were age and sex matched. Of the 35 patients 15 patients were studied after aspirin therapy. In these patients the study was done in two phases. The first phase was before the initiation of antiplatelet therapy and the second phase was after aspirin therapy in a dose of 50 mg/day for at least two weeks.

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Prior to blood collection, the patients should have been resting and not smoking. It was made sure that none of the patients had taken any medications known to interfere with platelet function, for at least two weeks prior to the test specimen collection.

A total of 10ml of blood was collected for the test, with 3.8% trisodium citrate as an anticoagulant in the ratio of 9 parts of blood to 1 part of anticoagulant. Specimens were kept at a temperature between 24° C and 27• C. The aggregation study was done within 20 minutes to there hours of collection of specimen. The instrument used was chronolog whole blood aggrometer model 540-VS. A platelet count was done on EDTA blood using the Sysmex 1000 counter.

In 10 patients, 15ml of blood was collected and PRP was prepared by the method of Dacie and Lewis. Platelet aggregation study was done on both whole blood and PRP for comparison.

Reagents used in the study

- Adenosine diphosphate (ADP), Collagen, Epinephrine , Thrombin
- A high concentration of ADP of 40 µm for a 1-ml sample.
- 10 µm concentration of collagen for a 1-ml sample was used.
- Epinephrine in concentration of $1x10^{-4}$ mol.1was used in the present study.
- Thrombin was used in a final concentration of 0.6units /ml.

Method

In the dual sample model the control and patients samples were run in parallel. Results were expressed as aggregation in ohms at a specific time interval from reagent addition.

Results

Platelet aggregation study was done on 35 patients of IHD. Of these 35 cases, 17 were patients of MI, 15 cases of angina and 3 cases of CIHD.
The youngest patient was 28 years old and oldest patient was 74 years old. The largest number of patients were in the age group of 45-64 years (62%) (Table 1).

Incidence of IHD was higher in males than females in the patients subjected to platelet aggregation study. Male to female ratio was 1.6:1. Among the 22 male patients, 14 had MI, 6 had angina and 2 patients had CIHD. Of the 13 female patients, 9 had angina, 3 had MI and 1 had CIHD.

Clinical features

The predominant complaint in almost all cases of MI was chest pain.

Platelet counts

Platelet count was conducted by sysmex counter for every case. Most of the patients had platelets ranging from 2 lacs / cumm- 4 lacs /cumm. Platelet rich plasma was prepared by standard method of Dacie and Lewis and the platelet count done thereafter tallied the expected counts ($2,50,000mm^3$-$4,00,000mm^3$).

Platelet Aggregation Study

The patients with IHD in this study had high aggregation response to all of the 4 reagents used, as compared to controls. The difference was statistically significant (Table 2).

Of the 35 patients studied, 33cases (94.2%) showed increased aggregation to ADP. Result of aggregation was 18.51+7.47SD. The present study showed a statistically significant increase in aggregation response to ADP in IHD patients as compared to controls (P value 0.0000). All the patients of IHD showed a pronounced increase in the platelet aggregation induced by collagen. The results were 25.21+ 7.08SD. This was again statistically significant.

In epinephrine induced aggregation, 27 cases (77.1%) showed a statistically significant increase in aggregation, 2 cases (5.7%) showed normal response and the remaining 6 cases (17.2%) showed no response. With thrombin, out of 35 patients 24 (68.8%) showed an increased response and 11(31.2%) did not show any response.

It was observed that platelet aggregation was high in both groups of patients- patients with MI and angina as compared to controls. The response was higher in cases of MI as compared to cases of angina and this increase in response was statistically significant with the reagent ADP and epinephrine. (P value 0.0080 and 0.0140 respectively).

Collagen and thrombin too showed an increased response in case of MI as compared to patients of angina, but the result was statistically insignificant (Table 3).

Out of the 35 patients, 15 were studied for the platelet aggregation after aspirin therapy in a dose of 50mg/day for a minimum period of two weeks. Patients on aspirin experienced a statistically significant reduction in platelet aggregation with all

the four reagents used (Table 4). Out of 15 patients, one showed a slight increase in platelet aggregation after aspirin intake. Another patient showed an increase in aggregation with only ADP, while the aggregation response with the other three reagents were significantly reduced.

There are very few studies on platelet aggregometric study on whole blood. In 10 patients out of total 35, platelet aggregation was done on whole blood and PRP for a comparative study. The aggregation response to agonists was higher in PRP than whole blood, however whole blood is equally effective as PRP in detecting hyperresponsive platelets in patients of IHD (Table 5). There was a statistically significant increase in aggregation in platelet rich plasma as compared to whole blood induced by collagen and ADP. Epinephrine and thrombin did not show a statistically significant increase in response with PRP.

Table 1: Age (in years) and sex distribution of Ischaemic heart disease

Diagnosis	25-34		35-44		45-54		55-64		65-75		Total
	M	F	M	F	M	F	M	F	M	F	
MI	1	0	3	0	3	2	3	1	4	0	17
Angina	0	0	0	2	2	5	3	2	1	0	15
CIHD	0	0	0	0	1	0	0	0	1	1	03
Total	1	0	3	2	6	7	6	3	6	1	35

Table 2. Platelet aggregation study in IHD patients and controls

Reagents	Patients (ohms/min)	Control (ohms/min)	P value
ADP	18.51±7.47	6.22±2.71	0.0000*
Collagen	25.21±7.08	8.97±4.04	0.0007*
Epinephrine	9.05±2.77	3.19±1.68	0.0000*
Thrombin	8.04±7.43	0	0.0000*

* = Significant

Table 3. Platelet aggregation study in MI and Angina

Regent	MI(ohms/min)			Angina(ohma/min)			P value
	Patients	Control	P value	Patients	Control	P value	
ADP	19.28±8.9	7.79±1.29	0.0000*	16.10±4.54	6.93±0.7	0.0000*	0.0080*
Collagen	27.9±7.54	12.0±1.78	0.0000*	21.63±5.87	9.43±4.1	0.0000*	0.1787**
Epinephrine	11.96±5.88	3.08±0.66	0.0002*	8.65±2.79	3.39±0.8	0.0000*	0.0140*
Thrombin	11.46±5.05	0	0.0004*	11.94±5.13	8.5±0.1	0.0014*	0.4281**

NB: * = Significant, **= Not significant

Table 4. Platelet aggregation study before and after aspirin in IHD

Regent	Before aspirin in ohms/min	After aspirin in ohms/min	P value
ADP	20.33±7.75	13.56±3.43	0.0051*
Collagen	25.66±6.1	19.4±4.7	0.0012*
Epinephrine	12.76±6.46	5.93±5.34	0.0006*
Thrombin	9.8±7.77	3.8±4.67	0.0016*

NB: * = Significant

Table 5. Platelet aggregation study in whole blood and PRP in IHD

Reagent	Whole blood (ohms/min)	PRP (ohms/min)	P value
ADP	19.45±5.98	26.05±6.86	0.006*
Collagen	24.55±6.32	31.25±9.93	0.00294*
Epinephrine	11.4±7.33	14.4±7.56	0.2759**
Thrombin	8.1±8.45	13.65±11.39	0.279**

NB: *= Significant, **= Not significant

List of Abbreviations

IHD - Ischemic Heart Disease
ADP - Adenosine diphosphate
PRP - Platelet rich plasma
MI - Myocardial infarction
CIHD - Chronic Ischemic Heart Disease
EDTA - Ethylenediamine tetraacetic acid
CAD - Coronary artery disease
5HT - 5 Hydroxytryptamine
AMI - Acute myocardial infarction

Discussion

Clinical and laboratory studies have shown that platelets play an important role in the pathogenesis of coronary artery disease (CAD).[3,4] Several investigators have noted platelet hyperaggregability in patients with myocardial ischaemia and infarction.[5,6] IHD can occur virtually at any age, but the frequency rises progressively with age.

The incidence of IHD in the present study was highest in the 6th decade of life with maximum number of cases observed between 45-65 years age group (62%).

In the present study a male preponderance in IHD was observed with a male to female ratio of 1.6:1. The incidence of IHD in female patients increased after menopause. This change in sex incidence with age is well known and the findings of this study have further substantiated this fact.

In our study, platelet aggregation was carried out on whole blood induced by 4 compounds i.e. collagen, ADP, epinephrine and thrombin in IHD patients and control subjects. Most of the patients showed increased aggregation response to all of the 4 reagents used, particularly in response to epinephrine. This finding suggests the potential for stress induced platelet activation and may be the cause of ischaemic symptoms in these patients.[7] The study by O. Brein et al[8] using collagen, ADP, epinephrine and 5HT on PRP have recorded increased platelet aggregation in

patients of MI. Zahaved el al[9] have also concluded that platelet aggregation started immediately without the initial rise in optical density and it reached a greater extent. The final disaggregation was minimal. Corresponding observations have been recorded in the present study using the whole blood aggregometer. Vilen et al[10] have studied ADP induced platelet aggregation in young female survivors of MI. There results were similar to the present findings of enhanced platelet reactivity in patients with documented heart disease. Similarly, Ardlies[11] findings of increased platelet aggregation in MI, corroborates with the present study.

The present study demonstrated that all the patients showed increased response to collagen. 33 patients showed increased response to ADP, while two showed normal response. Epinephrine showed increased response in 27 patients, six showed no response and two patients showed normal response. Thrombin induced aggregation was increased in 24 patients, the remaining 11 showed no response.

Steele et al[6] observed abnormally high aggregation to collagen and ADP in only two patients. Five had borderline high values and the rest showed normal aggregation. In contrast to the present study most of the patients in their study showed a normal response. Similarly Salky N et al[5] did not get an abnormal response to ADP in their patients but most had persistent marked hyperresponsive platelets to collagen, which lasted for months.

It has been theorised that platelet behaviour may be largely governed by plasma factors.[12] An alpha globulin that inhibits collagen induced platelet aggregation has recently been found in normal plasma. Most probably, a lack of this factor results in the platelet hyperaggregability to collagen seen in patients of IHD.

In our study, increased platelet aggregation to various agonists was observed in patients of MI and angina. Neri Serneri el al[13] have reported increased platelet aggregation of the same magnitude inpatients with remote MI, spontaneous angina and effort angina when compared with controls. They concluded that patients with frequently occurring clinical symptoms show increased platelet aggregation compared to those with more quiescent symptoms. This conclusion was supported by the findings of Sorkin el al.[14] They found the highest in vivo aggregation by filtagometer technique in patients with AMI followed by patients with recent history of AMI and or angina pectoris. These findings are comparable with the present study where increased platelet aggregation was observed in MI and angina, with higher values in the former.

Antiplatelet therapy particularly with aspirin, and its ability to decrease platelet aggregation has become the focus of research in the treatment and prevention of cardiovascular disease. In the present study the effect of aspirin at a daily dose of 50mg/day was evaluated in 15 of the 35 patients. The study showed a significant reduction in platelet aggregation with all the four reagents although the values did not come down to control levels.

There are few studies conducted on platelet aggregation in IHD after aspirin intake. Hennekens C.M. et al[15] had done an observational study to determine the relationship between IHD and use of aspirin. The results were compatible with small to moderate benefits of antiplatelet therapy. Eldwood el al[16] too have shown a beneficial effect of aspirin in the primary and secondary prevention of IHD. Similarly Jick et al[17] found a protective effect of aspirin.

Most of the platelet aggregation studies done so far have been on PRP by the turbidometric method utilizing the optical aggregometer and is based on the turbidometric method of Born.[18] It has many disadvantages.[19] These include sample preparation by centrifugation which removes the other cellular elements and a proportion of heavier platelets from the final sample. RBCs & WBCs may influence platelet aggregation. To minimize these disadvantages platelet aggregation has recently been studied by means of whole blood aggregation technique by the impendence aggregometer .[20]

In our study, platelet aggregation studies were done on whole blood and PRP in 10 patients for a comparative study using aggrometer model 540-VS. It was found that although whole blood was equally effective as PRP in detecting hyper-responsive platelets in patients of IHD, platelet aggregation response was higher in PRP as compared to whole blood with similar concentration of reagents.

Conclusions

The present study showed that platelets are hyperaggregable in patients of ischaemic heart disease and this hyper-responsiveness is reduced with aspirin therapy. Platelet function studies should be employed more widely in patients with ischaemic heart disease especially in those who do not manifest any arteriographic lesions, as antiplatelet therapy will help in bringing down the mortality and morbidity in them.

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