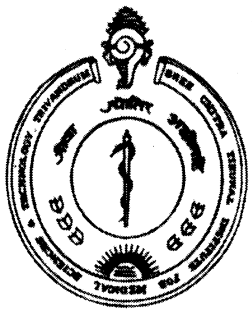

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY

THIRUVANANTHAPURAM - 695 011

PROJECT REPORT

Name	:	**SYAM KUMAR M.D**
Programme	:	**D M Cardiology**
Month & Year of Submission	:	**June 2001**

CERTIFICATE

I **Dr. SYAM KUMAR** hereby declare that I have actually carried out the two projects under report.

Signature.....

Place : Trivandrum

Name in capital letters

Date : 19.6.01

SYAM KUMAR

Fowarded. He has carried out the two projects under report

Signature
Head of the Department

SREE CHITRA THIRUNAL INSTITUTE FOR MEDICAL SCIENCE AND TECHNOLOGY TRIVANDRUM - 695 011	NAME	
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PROJECT WORKS DONE

1. FUNCTIONAL OUTCOME AND FOLLOW UP ASSESSMENT AFTER ATRIAL SWITCH PROCEDURES FOR TRANSPOSITION OF GREAT ARTERIES

2. EVALUATION OF EFFICACY OF ASPIRIN AS AN ANTI PLATELET AGENT IN PATIENTS WITH CAD USING PLATELET AGGREGATION STUDIES

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PROJECT REPORT

(PROJECT No. 1)

TITLE

FUNCTIONAL OUTCOME AND FOLLOW UP ASSESSMENT AFTER ATRIAL SWITCH PROCEDURES FOR TRANSPOSITION OF GREAT ARTERIES

Name : **SYAM KUMAR M.D**

Programme : **D M Cardiology**

Month & Year of Submission : **June 2001**

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SREE CHITRA THIRUNAL INSTITUTE FOR
MEDICAL SCIENCE AND TECHNOLOGY
TRIVANDRUM - 695 011

FUNCTIONAL OUTCOME AND FOLLOW UP ASSESSMENT AFTER ATRIAL SWITCH PROCEDURES FOR TRANSPOSITION OF GREAT ARTERIES

SYAM KUMAR M.D.

FUNCTIONAL OUTCOME AND FOLLOW UP ASSESSMENT AFTER ATRIAL SWITCH PROCEDURES FOR TRANSPOSITION OF GREAT ARTERIES

Surgical treatment of patients with simple and complex d-transposition of great arteries has improved remarkably over last 2 decades with options of atrtal switch procedures- Mustard and Sennings operation or an arterial switch. The latter procedure was originally designed for patients with TGA with VSD where mortality of alternative procedures has remained high, but presently performed successfully in neonates with simple TGA. This opertaion avoids late RV dysfunction and Tricurpid regurgitation, however early mortality rate of arterial switch procedure remains approximately 10-17.5% at best.

Atrial repair was first introduced by Senning in 1959 and based on rerouting the systemic venous return through the mitral valve and pulmonary venous return though tricuspid valve[1]. This type of repair involves extensive atrial procedures which can lead to atrial arrythmias and obstruction of venous pathways; and requires anatomic right ventricle to sustain systemic circulation for life with the potential for late ventricular dysfunction[2,3]. Many long term studies have highlighted this phenomenon.[4,5,6,7,8,9]

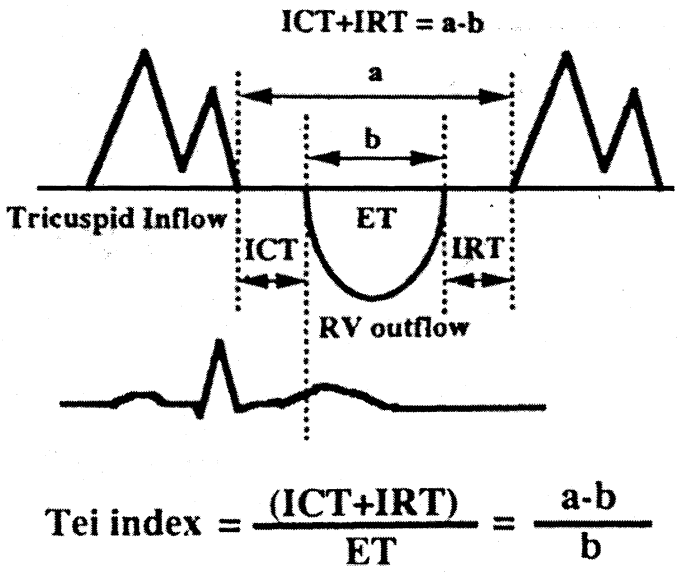
Even though arterial switch operation is the procedure of choice in patients with TGA, not all patients are candidates for arterial switch. Neverthless, atrial repair has its place in selected patients with special coronary artery patterns[10].

Many studies on RV function following Sennings procedure have either utilised angiography or Radio nuclide angiography as a tool for measuring RV ejection fraction[4,6,11].There has been studies utilising2 D echo cardiography in assessment of RV function and other

parameters[11,12,13]. There has been good agreement between echo cardiography and MRI in assessment of ventricular function in TGA patients treated with Sennings operation[13].

Right ventricular performance can be assessed by Doppler methods also. Aortic blood flow acceleration by pulsed Doppler ultrasound has been used in patients with atrial switch[14].

Global right ventricular function can be evaluated by an index initially proposed by Tei et al, while is simple, reproducible and independent of heart rate BP, degree of TR and severity of PAH[15]. This index being a time ratio, it is independant of ventricular geometry and particularly useful for evaluating RV function in children with congenital heart disease and complex RV morphologies.



Schema of Doppler time intervals. The Tei index is calculated as *(a - b) / b*, where *a* is the interval between cessation and onset of the tricuspid inflow, and *b* is the right ventricular ejection time. *ICT*, isovolumetric contraction time; *IRT*, isovolumetric relaxation time; *ET*, ejection time; *RV*, right ventricle.

AIM OF STUDY

Purpose of this study was to determine the functional outcome of patients who underwent atrial switch procedure for TGA who survived after the immediate postoperative period and to evaluate the cardio vascular status of those patients.

MATERIALS AND METHODS

41 patients who survived immediate post operative period (within 30 day) after atrial switch Mustards or Senning) procedure at SCTIMST Trivandrum were identified. Date of surgery ranged from 1978 to 2000; cross sectional follow up of survivors were taken between November 2000 to April 2001. Patients who had died during immediate post op period were excluded from this analysis.

Medical records, preoperative echocardiogram, cardiac catheterisation data and operative notes were reviewed to obtain the data on pre operative& perioperative variables.

Outcome measurements

Following assessments were done during cross sectional follow up period.

New york Heart association(NYHA) classification of functional class

Physical examination, pulse oxymeter saturation

Electro cardiogram-12 lead with rhythm strip

Holter evaluation when 12 lead ECG suggests rhythm disturbance

Treadmill evaluation for Effort tolerance in grown uppatients and those who complained of symptoms.

Echo cardiographic evaluation

Follow up period ranged from 2 month to 23yrs with a mean of

7.98yrs (7.98 $\pm$ 4.97 years)

Echo Cardiographic evaluation

All children were subjected to echo cardiography. Echo cardiography done by using sonos 1000 (Hewlett Packard) and Ving med system V machines. Both 2.5 MHz and 5.0 MHz transducers used whereever appropriate. 2D evaluation, color Doppler evaluation and spectral Doppler evaluation done.

2D ejection fraction of right ventricle was calculated by using simpson's formula $V= \pi/3\ hxD_1D_2$. Views chosen were apical 4 chamber subcostal and parasternal short axis views. This method had been validated against radio nuclide method by Vitolo et al and against MRI assessment. 3 cycles were chosen and average of 3 cycles were taken as mean EF.

For pulsed Doppler evaluation, recordings of aortic blood flow spectral recordings. Following variables are measured and calculated from these tracings-peak and mean aortic flow Acceleration time (AT) ejection time (ET). Acceleration time and ejection time were measured from onset of ejection to point of peak velocity (PKV) and to end of ejection respectively. Each Doppler measurement was repeated in 3-5 consecutive cardiac cycles and averaged.

RV Tei index calculation from doppler interrogation of tricuspid and aortic valves. Tricuspid inflow velocities are recorded from apical 4 chamber or apical long axis view with pulsed Dopplar sample volume positioned at tips of tricuspid leaflets using diastole. Subsequently RV out flow velocity pattern was recorded with Doppler sample volume positioned just below aortic valve. Since the 2 measurements are not simultaneous, cardiac cycles taken with variability $<5\%$ in cycle length.

RV ejection time calculated from onset to end of RV out flow spectral recording.Tricurpid valve closing and opening was measured

as interval from the end to the onset of tricuspid inflow. Mean values were obtained by averaging at least 5 sequential beats- scheme of measurement shows in figure.

RV diastolic function assessment done with tricupid valve inflow pattern and pul.venous inflow interrogation.

TAPSE : Tricupid annular plane systolic excursion. TAPSE is a simplified assessment of right ventricular ejection fraction TAPSE is measured in apical 4 chamber view (16,17). This measurement has been found to correlate well with radio nucliede ejection fraction. M-mode of tricuspid annulus which not only quantitates excursion but also provides temporal appreciation of the right ventricular annulus motion[17].

Pulmonary venous channel evaluation

Stenosis of common pulmonary venous channel after Sennings operation is a serious problem. The pulmonary venous channel diameter can be measured in 4 chamber view. Short axis views of left ventricle is useful in evaluating pul.venous channel stenosis. When the ventricle is flat shaped and pul.venous channel diameter > 10mm, significant obstruction is unlikely. Round shape of LV and PV channel < 6mm is suggesture of significant obstruction[18].

Statistical analysis For assessing statistical, one way ANOVA and Chi.square tests were used. P.values of < 0.05 were considered to be of statistical significance.

RESULTS

Study consisted of 41 patients s who survived the immediate post operative period after atrial switch operation. There were 30 males (72.5%) and 11 (27.5%) female. Mean age was 9.87 yr ± 5.99 at the time of examination- (range 1.5 to 33 yrs).

38 patients underwent Sennings procedure (92.5) and 3 (7.5%) patients, Mustards operation. Age distribution among late survivors.

Age	No	%
0-5	10	24.39
6-10	9	21.95
11-15	19	46.34
16-20	2	4.1
>20	1	2.4

Anatomic Sub types

Diagnostics group	No	%
Simple transposition	31	75.6%
Complex transposition	10	24.4%

Balloon atrial septostomy

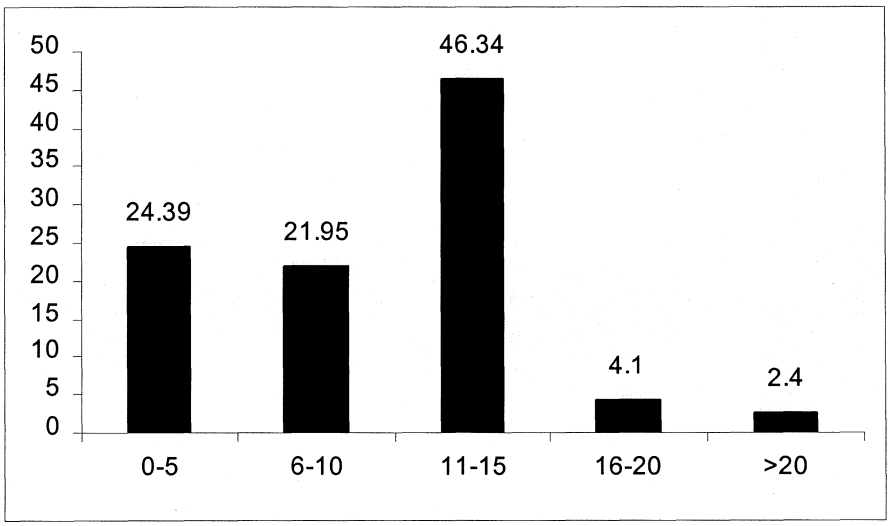
BAS performed in 35 cases - 85.36%
Not dose in 6 cases - 14.6%

Systemic arterial saturation Pre BAS : 40.20% ± 13.90
(Range 20% to 75%)

Saturation improved post BAS - Mean value 71.12 ± 9.46
(Range 51 - 88%)

Mean age of BAS in days - 56.16 ± 50.44 (Range 2 days to 165 days)

AGE DISTRIBUTION



Mean age at operation 1.89 $\pm$ 2.11 yrs (Range 4 months to 10 yrs)

CPB time was 126.97 min $\pm$ 19.23 (Range 60 - 171)

Aortic clamp time 70.91 $\pm$ 12.48 min(52 - 114)

Followup assessment

Symptom class - 40 of 41 patients were in symptom class I NYHA (97.5%). Patient who had class II symptoms underwent exercise stress test and had very good tolerance of 13 mets

Systemic saturation

39 of 41 patients had normal systemic saturation (95%) 2 patients had mild systemic desaturation - of 87%. One of which had residual pulmonary stenosis and a baffle leak from systemic venous channel to pulmonary venous channel

Analysis of ECG

All ECGs were analysed with particular emphasis on arrhythmias. 39 patients (95%) where in normal sinus rhythm. One (2.5%) was having sinus rhythm with frequent atrial ectopics. One patient was in junctional rhythm. One among the 39 patients who were in sinus rhythm at time of evaluation had incessent PAT which required cardioversion to sinus rhythem.

Stress testing done in 2 patients with Bruce protocol tread mill test. Both had good effort tolerance of > 10 mets.

Chest Radiographs

Average CTR : 51.21 + 4.03 (45 to 63%)

Holter evaluation : 2 (5%) underwent Holter evaluation which didnt show significant pauses or tachycardia requiring treatment.

Rt ventricular function

Patients were categorised to 3 groups according to RV ejection fraction

Group I RVEF < 45%
II RVEF 45 - 55%
III RVEF >55%

Group	No. of pts	%
I	4	9.7%
II	12	29.26%
III	25	60.97%

Tei index : showed good correlation with RV ejection fraction

Group	Tei index
Group I	0.73 ± 0.21
Group II	0.73 + 0.29
Group III	0.49 + 0.49

$p < 0.05$

Aortic flow Doppler evaluation acceleration / ejection time ratio

Group	Ratio
Group I	0.38 + 0.096
Group II	0.34 + 0.042
Group III	0.27 + 0.055

p value 0.0003

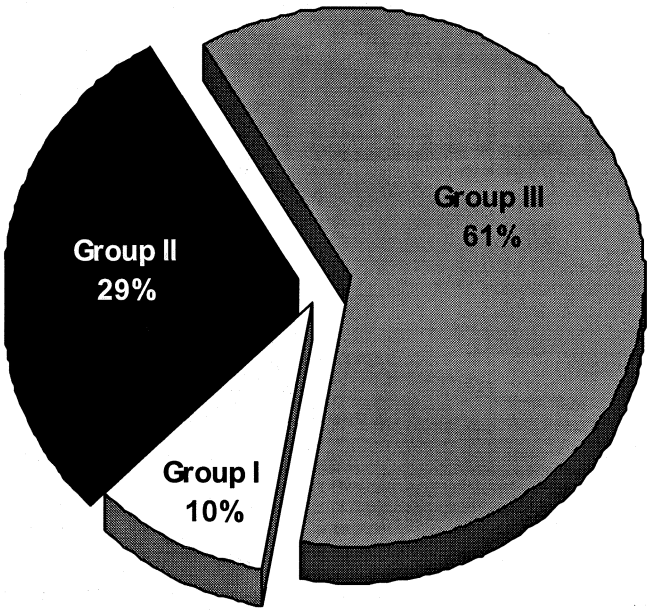
TAPSE

Also showed correlation with RV performance

Group	TAPSE(mm)
Group I	12.22 ± 2.19
Group II	13.08 ± 1.54
Group III	17.78 ± 1.98

$p < 0.05$

SYSTEMIC VENTRICULAR FUNCTION (EF %)



CTR

Group	CTR
Group I	54.75 + 4.11
Group II	53.70 + 4.39
Group III	49.61 ± 2.99

p 0.0034

Tricuspid regurgitation

Group	TR			Total	%
	Trivial-mild	Moderate	Severe		
Group I	4	1	0	5	15.4%
Group II	6	1	0	7	26.9%
Group III	14	1	0	15	57.7%

TR and RV function did not have significant association - p 0.19726

Baffle obstruction

Mean baffle diameter was 12mm ± 2 mm.
6 patients had baffle obstruction - mild baffle obstruction

Group I	1	25%
Group II	2	16.6%
Group III	3	12%

Baffle leak

3 patients had baffle leak

1 had pul. venous to systemic venousbaffle leak

2 had systemic to pulmonary venous baffle leak

Group I	0	0%
Group II	2	16%
Group III	1	0.4%

One patient baffle obstruction had class II symptoms.
Baffle leak didnot have any correlation with RV performance/NYHA class
Desaturation was present in a patient with leak to pulmonary system in one patient

Variables correlated with functional status of systemic ventricle

Of the variables analysed

age at BAS, surgery, followup duration, saturation, anatomical subtypes of TGA, CPB time or ACC time were not determinants of systemic ventricular function ($p > 0.05$)

RV performance index, AT / ET ratio, TAPSE and CT Ratio in CXR correlated well with RV EF measured by modified Simpson's method $p < 0.05$.

DISCUSSION

This study details the functional and anatomical status of patients who underwent atrial switch operation for d-TGA. Most patients (97.5%) perceived their state of health to be good and class I; and were able to participate in normal activities.

The methods of atrial correction have a principal draw back in that they employ the right ventricle as systemic ventricle. Experience in patients with congenitally corrected TGA shows that heart, failure may occur after 3rd, 4th, 5th decade of life but this phenomenon has been observed mostly in large VSDS. Further more, in congenitally corrected transposition, systemic AV valves seem to fail in later life[19]. Otherwise healthy patients with congenitally corrected transposition might have no functional deterioration. One syudy showed the failure of systemic ventricle late after corrective surgery systemic ventricular failure was more in complex TGA with complex anomalies and VSD[21]. Present study didnt show any significant correlation between the anotomical subtype and SV dysfunction. All patients with TGA may show a deleterious effects of cyanosis on the myocardium. In our study there was no clear cut association between the age at BAS or correction and ventricular dysfunction. Several other theories have been put forward for explanation for SVF, such as a mismatch between right ventricular blood supply and demand[22]. The Rt ventricle also seems unable to adapt to the situation by developing primary hypertrophy. The disturbing fast remains that occurance of SVF is relatively unpredictable. Many patients after correction of TGA lead normal life without signs of heart failure. Incidence of SV dysfunction varies from 0 - 10% (23 - 25).

Yet another important complication after atrial correction of TGA is serious rhythm disturbance. Turina et al reported sudden death in 7 patients in the followup study of 220 operated cases. Significant sinus node dysfunction necessitating pacemaker implantation was noted years in 7% at 10yrs[21]. Present study at a

mean followup of 7.98 years, only one patient had features of sinus node dysfunction, but was asymptomatic &Holter evaluation showed no significant pauses requiring pacemaker implantation. The incidence of rhythm disturbance seems to be decreasing in recent years as more experience is gained with atrial correction of TGA.

Atrial correction provides excellent symptomatic and functional improvements. Genoui et al[26] from Zurich reported extended follow of 239 pts .All the surviving patients werein class I.

Birnie et al report 15 yrs followup of 131 patients who underwent correction of TGA and 71.3% Survival at 15 yr. There was significant incidence of late cardiac deaths and events. They concluded sennings procedure preferable to Mustard for cases unsuitable for arterial switch.[27]

In summary, atrial correction of TGA given good functional results in majority of patients and has low incidence of systemic ventricular dysfunction or arhythmias. Still late arrhythmias and SV failure remains of some concern. SVF doesnot seem to have a single cause, and only further experience will tell if the incidence of complication can be reduced by one of the modern methods of myocardial protection.

Limitations

This cross sectional study included only patients surviving the early post op period. Those died in the immediate post period were not included. So the prediction of perioperative risk factors of a successful surgery could not be assessed.

CONCLUSION

At mean follow up of 7.89 yr, Atrial switch operation provides excellent functional outcome. Incidence of systemic ventricular dysfunction is relatively low, Arrhythmias are extremely rare and almost all patients are in symptomatic class I. There were no late deaths Atrial switch procedure is a good alternative procedure for rare cases where the use of arterial switch procedure in limited.

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PROJECT REPORT

(PROJECT No. 2)

TITLE

EVALUATION OF EFFICACY OF ASPIRIN AS AN ANTI PLATELET AGENT IN PATIENTS WITH CAD USING PLATELET AGGREGATION STUDIES

Name : **SYAM KUMAR M.D**

Programme : **D M Cardiology**

Month & Year of Submission : **June 2001**

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EVALUATION OF EFFICACY OF ASPIRIN AS AN ANTI PLATELET AGENT IN PATIENTS WITH CAD USING PLATELET AGGREGATION STUDIES

SYAM KUMAR M.D.

EVALUATION OF EFFICACY OF ASPIRIN AS AN ANTI PLATELET AGENT IN PATIENTS WITH CAD USING PLATELET AGGREGATION STUDIES

Knowledge and research into platelet-vessel wall interaction and the steps that lead to thrombus formation should result in a better understanding of management strategies in patients suffering from vascular events. The introduction of various drugs that are able to block platelet metabolic pathways and coagulation pathways and development and use of anti platelet agents has been the result of an increased understanding of pathways involved.

The platelet rich intra coronary thrombus is central to the pathogenesis of acute MI, unstable angina and majority of complication of percutaneous coronary interventions. Until recently, aspirin was the only drug to prevent or treat these events. If intra arterial thrombus formation could be prevented, the major sequelae of atherosclerotic heart disease as well as majority of complications of PCI could be prevented.

Intra Coronary thrombosis

Intra coronary thrombosis begin with disruption of endothelial monolayer either spontaneously or because of a mechanical device such as a balloon or stent leading to a monolayer of platelets adhering to the newly exposed subendothelium. Adhesion is mediated by binding of platelets to normally hidden subendothelial glycoproteins such as von Willebrand factor, fibronectin and collagen.[1,2]

Platelet activation also initiates the platelet release reaction, which entails the freeing of pre packaged granules containing ADP, serotonin,

fibrinogen and other compounds that then promotes feather platelet activation(3,4) Once activated, a conformational change occurs in platelet glycoprotein (GP) IIb/IIIa receptor, the exclusive mediator and final common pathway of platelet aggregation, which allows for the formation of a platelet aggregate.

Thromboxane and ADP released from the activated platelets leads to the activation of surrounding platelets thereby amplifying the thrombotic process. Activated platelets accelerate the production of thrombin in the coagulation cascade, which then stabilize the thrombus by conversion of fibrinogen to fibrin as well as potentially promote further platelet activation.[4]

Aspirin

Aspirin has been available commercially over 100 yrs and was the 1st pharmaceutical agent available in pill form. The clinical benefits of Aspirin therapy in the prevention of thrombotic complications of cardiovascular disease have widely been documented.[5] Among all subgroups of patients, aspirin decreases the risk of a vascular event by about 25% compared with placebo. The level of absolute benefit is greatest in those who are at highest risk of vascular events, eg: those with AMI, unstable angina and least in low risk population, such as recepients of primary prevention. This 25% reduction in vascular events from aspirin highlights the importance of anti platelet therapy for the prevention of cardiovascular morbidity and mortality and makes asprin the most cost effective pharmacologic intervention available today. It is important to evaluate, not why aspirin decreases the events by 25%, but rather why it does not prevent vascular events in 75% of at risk population. All patients have been assumed to achieve similar anti thrombotic protection with a set dose of aspirin. This assumption is partially related to difficulty associated with measuring specific patient

response to aspirin therapy. A number of studies including a total 975 patients and using different methods for determining response to aspirin revealed a substantial degree of inter patient variability [6-13]. 8 - 60% of the patients studied in the above studies were considered particular non responders to asprin.

Biological effects of aspirin is measured by the inhibition of platelet aggregation in patients taking aspirin for secondary prevention of CAD.

AIM OF STUDY

Present study was undertaken to evaluate the exvivo effect of aspirin on platelet aggregation: 1, To determine the aspirin response or failure in patients with coronary artery disease and 2) to evaluate the effects of 2 different doses of aspirin on platelet aggregation.

MATERIALS AND METHODS

3 groups of population (total No : 102) were included in study, namely group I (No:33) patients with documented coronary artery disease who are on 162.5 mg of dispersible aspirin for at least 48 hrs. Group II: Patients without clinical evidence of vascular disease and not exposed to aspirin or other anti platelet agent.

Group III : Patients with known coronary artery disease who are on cardiac drugs except aspirin - Patients who are waiting for CABG in whom aspirin has been withdrawn for > 10 days

Inclusion criteria : All patients were informed about study

Exclusion : Those who are on other anti platelet drugs and / or anti coagulants

Group I

1. Patients with documented coronary artery disease by CAG
2. Patients on soluble aspirin 162.5 mg for atleast 48 hours.
3. Age > 35 years

Group II - Control

1. Age > 30 year
2. No clinical evidence of coronary artery disease or other vascular diseases.
3. No conventional coronary risk factors requiring medical treatment
4. Sinus rhythm

Group III

1. Documented coronary artery disease by CAG
2. Aspirin has been stopped > 10 days
3. On other cardiac drugs

Blood Collection :

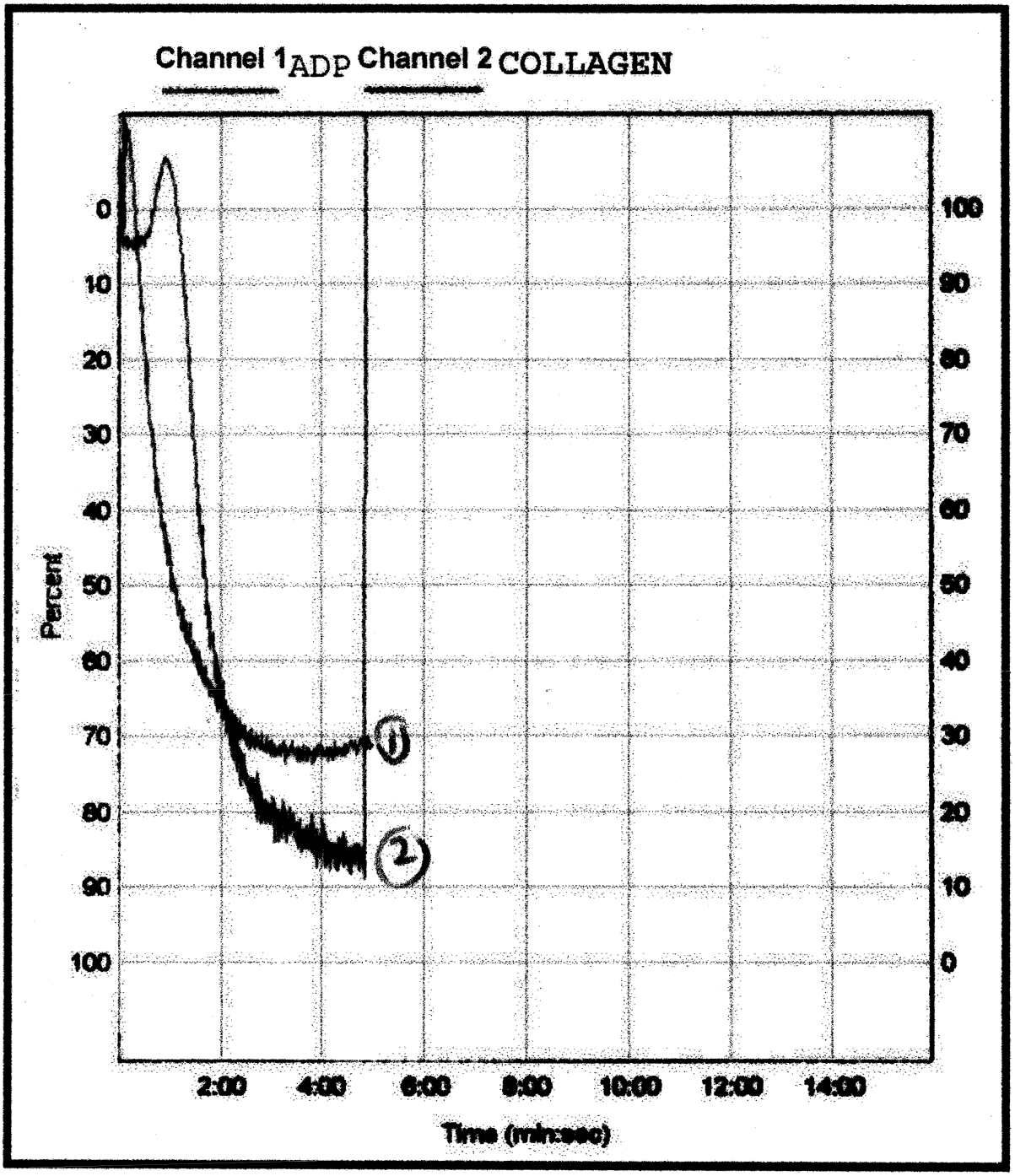
The schedule for blood collection and conduction of the test were done within 1 - 1½ hours. All tests were done in the morning to avoid circardian variation.(14), A 22G needle and plastic syringe was used for obtaining blood sample. 1.5 ml of ACD and 8.5 of whole blood were immediately mixed in a poly styrene (PS) Falcon tubes.

Preparation of Platelet Rich Plasma (PRP) and Platelet Poor Plama (PPP)

Blood was centrifuged at 300g in a Heraeus Labofuge 300 centrifuge (Heraeus Corp. W. Germany) using RPR - 10, swingout bucket rotor between 20^0 and 24^0 for 5 minutes. PRP was removed and remaining blood was centrifuged at 600 g for 10 min. and PPP was collected. Platelets in PRP and blood in 1.5ml of EDTA was counted on an automatic haematology analizer COBAS MINOS vet model (Roche Riagnostices, France) and mean platelet volume, platelet crit and platelet histograms were taken.

Platelet aggregation :

Aggregatory response of platelets were studied using a Chronology 560Ca^{2+} whole blood Lume - aggregometer (Chronolog Corpn. USA) Agonists used were adenosine diphosphate (ADP) at concentrations of 10µmol/l and 20µmol/l and sonicated insoluble collagen type II 5 µgm/ml, 10 µgm/ml. All reagents were obtained from Sigma chemicals co (St. Louis USA) Parameter measured were amplitude of aggregation and (% transmission) and slope of aggregation (rate of increase in % transmission with time) The computer software Aggrolink obtained from Chronolog computed the data on each aggregation test to obtain the amplitude and slopes. Platelet count in PRP is adjusted to 2,50,000/ml using PPP.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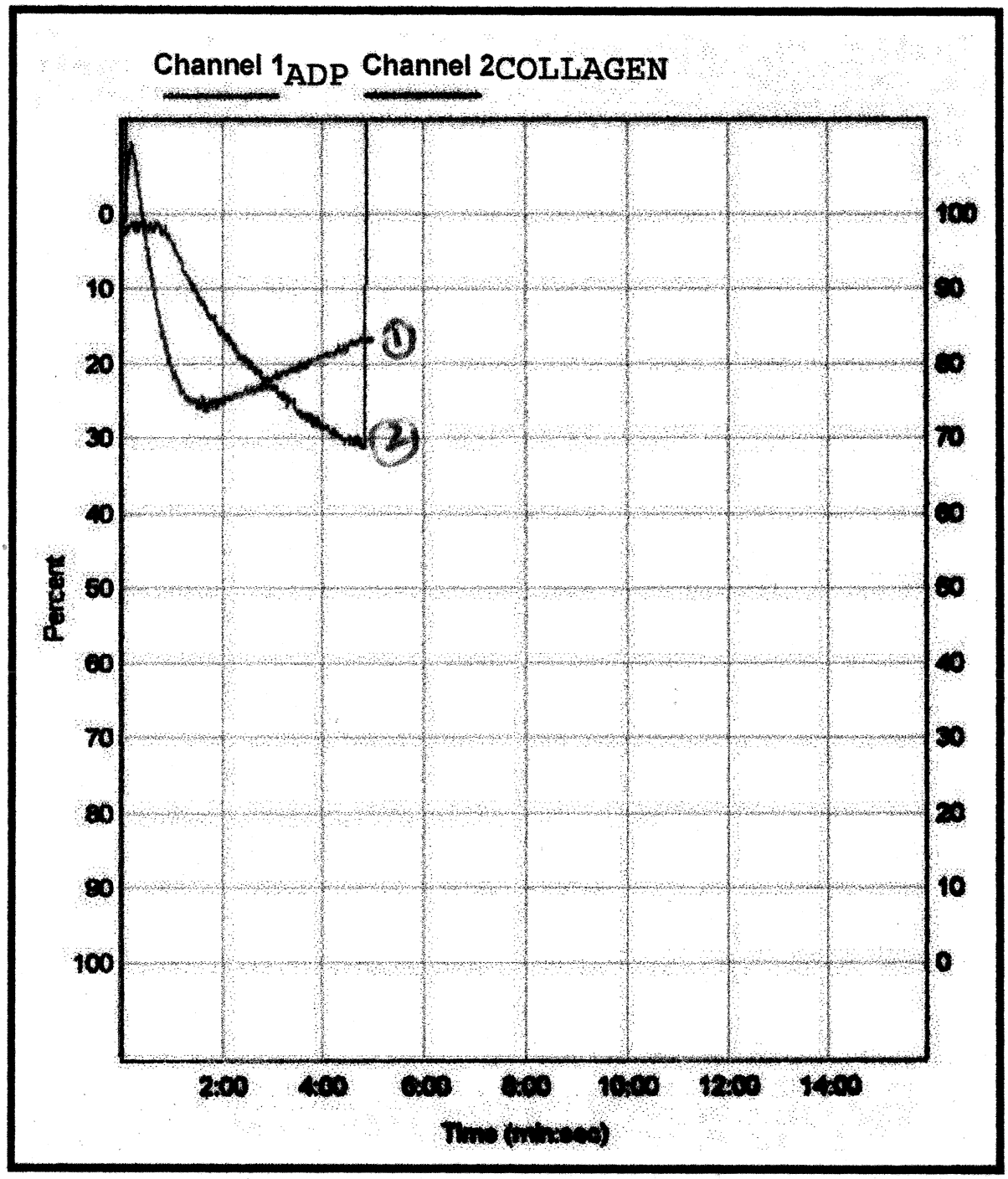
14:00
Time (min:sec)
1
2

Graph 2 (Aggregation curves of aspirin group) (



ADP responses :

ADP responses are only partly dependent on intact cyclooxygenase, and ADP gives dose dependent responses whether or not the patient is treated with ASA.

Collagen response

Collagen induced platelet aggregation is primarily dependent on intact cyclo oxygenase. Collagen induced aggregation is considered to be absent at $< 10\%$ aggregation (90% inhibition) markedly decreased at 10 - 20% aggregation, decreased to at $< 50\%$ aggregation, slightly decreased at $> 50\%$ - 65% aggregation.

Aspirin treatment was considered fully effectives when following were observed.

1. ADP response is slightly, moderately or markedly decreased
2. Collagen response is absent or significantly decreased.

Asperine treatment was considered less than fully effective (partly inhibiting platelet function) when ADP response was completely normal, collagen response was normal or only slightly decreased.% inhibition calculated using formula

$$\% \text{ inhibition} = 100 - \left(\frac{\text{amplitude/slope of cases}}{\text{amplitude/slope of control}} \times 100 \right)$$

Statistical analysis

Analysis was done with SPSS software. Values are noted as mean $\pm$ SD. Statistical significance was obtained using student 't' test $P < 0.05$ is considered significant.

Baseline characteristic of 3 groups : (n : 93)

Table I

	Group I n :33	II n:35	III n:25
Male / female	28/5	21/14	24/1
Age (Yr)	53.1 a± 11.73	39.46 ± 15.06	49.77 ± 7.49
Current smoking	2	-	-
Hypertension	25	6	20
Diabetes	13	1	8
Total Cholesterol	203 ± 37.9	180.78 ± 28.3	190.78 ± 18.4
Baseline medications			
Nitrates	33	-	25
Betablockers	28	2	24
ACEI	27	3	23
CCB	23	2	21

Table II

Quantitative variable of platelet function

	Group I	Group II	Group III
platelet count ($X10^2$/ml)	196.193	197.060	180.50 + 37.55
$X10^2$/ml	± 52.14	±63.06	
platelet crit (%)	0.139 ± 0.033	0.1357 ± 0.038	0.1306 ± 0.022
Mean platelet volume (pl)	7.08 ± 0.70	6.97 ± 1.12	7.16± 0.586
Platelet distribution width	18.03 ± 1.24	18.57 ± 2.14	18.08 ± 1.45

Table III
platelet aggregation in all group

	Group I Aspirin on	group II control	group III Aspirin withdrawn
ADP			
% transmission (Amplitude)			
10 µmol	38.00 ± 15.64	98.97 ± 16.81	45.63 ± 13.80
20 µmol	45.05 ± 21.33	51.03 ± 0.03	50.72 ± 16.75
Rate of Increase in % transmission with time (slope)			
10 µmol	55.25 ± 17.82	68.40 ± 1789	70.63 ± 17.59
20 µmol	61.08 ± 20.32	71.00 ± 19.25	70.22 ± 21.07
Collagen			
Amplitude			
5 µgm	37.75 ± 19.94	65.11 ± 19.81	64.77 ± 15.51
10µgm	44.87 ± 20.60	67.57 ± 20.61	67.04 ± 14.36
Slope			
5µgm	35.53 ± 24.91	73.68 ± 23.78	83.59 ± 21.73
10µgm	39.21 ± 24.11	76.51 ± 24.87	84.18 ± 19.37

Quantitative assessment of platelet

Platelet count in patients on Aspirin was not significantly different from control group 196.19 ± 52.14 × 10^3/ml, 197.06 ± 63.06 × 10^3/ml 180.50 ± 37.55 ×10^3/ml P > 0.05, likewise, platelet crit in patients on aspirin was 0.139 ± 0.033, in control it was 0.135 ± 0.038 and in drug withdrawn patients it was 0.1306I 0.022 (P : NS)

Mean platelet volume (7.08 ± 0.70, 6.97 ± 1.12, 7.16 ± 0.586 respectively) P (NS)

platelet distribution width also was similar in all groups (18.03 ± 1.24, 18.57 ± 2.14 and 18.08 ± 45) respectively (P.NS)

Aggregation studies

Platelet aggregation in patients on aspirin was significantly lower compared to controls and drug withdrawn groups

Amplitude and slope of aggregation curves were significantly lower in aspirin group.

Amplitude collagen 5 µg/ml

group I : 37.75 ± 19.94 P <0.001

II : 65.11 ± 19.81

% inhibition : **42.02%**

Slope - 5µgm collagen

group I : 35.51 P < 0.001

II : 73.68

% inhibition : **51.80%**

Percentage inhibition with collagen 10 µgm

Amplitude : group I. 44.87 ± 20.60 P < 0.001

II.67.57 ± 20.61

% inhibition : **33.59%**

Slope : group I. 39.21 ± 24.11 P < 0.001

II 76.51 ± 24.87

% inhibition : **48.75%**

Percentage inhibition with ADP

Slope of ADP aggregation curves :

group I 55.21 ± 17.82

10 µ mol II.68.40 + 17.89

% inhibition = **19.22%**

Amplitude of aggregation curves

I = 38.00 + 15.64 P 0.027

II = 48.97 + 16.81

% Inhibition = **32.40%**

Percentage inhibition with ADP 20 µmol

Amplitude I. 45.65 + 21.33 P. 0.193

II.51.03 + 0.03

% Inhibition = 11.71%

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Percentage inhibition with ADP 20 µmol

Amplitude I. 45.65 + 21.33 P. 0.193

II.51.03 + 0.03

% Inhibition = 11.71%

Slope

group I 61.08 + 20.32
II 71.00 + 19.27 P = 0.43

% Inhibition = **13.97** %

Thus, there is significant inhibition of platelet aggregation induced by collagen and modest inhibition of aggregation induced by ADP by 165.5 mg Aspirin.

Comparison of 2 doses of Aspirin - 165.5 VS 325 mg

A sub group study was conducted to evaluate platelet response to 2 doses of Aspirin. 162.5 mg and 325 mg. in same individual. Quantitative variables of platelet function eg: platelet count, platelet unit, mean platelet volume and platelet distribution width were almost similar with 2 doses of aspirin.

Aggregatory parameter with 2 doses of aspirin evaluated with collagen and ADP.

Results of aggregation studies using collagen.

with 5 μgm of collagen, Amplitude of aggregation curves.

Single dose : 39.200 + 14.94 P = 0.472
Double dose : 43.72 + 16.45

Slope

Single dose : 33.733 + 22.15 P= 0.936
Double dose : 33.09 + 16.24

Amplitude : collagen 10μgm

Single dose : 51.68 + 12.86 P = 0.172
Double dose : 59.81 + 17.19

Slope :
Single dose : 45.50 + 20.77
Double dose : 46.45 + 20.89 P = 0.908

ADP aggregation curves
ADP - 10 µmol - Amplitude
Single dose : 52.73 + 16.27
Double dose : 58.81 + 23.39 P = 0.441

Slope
Single dose : 71.46 + 18.60
Double dose : 77.72 + 23.80 P = 0.458

Aggregometry with 20 µmol

Amplitude
Single dose : 55.62 + 13.70
Double dose : 66.81 + 24.28 P = 0.220

Slope
Single dose : 75.00 + 18.26
Double dose : 77.36 + 24.63 P = 77

Even though aggregation is not changing to an extent of statistical significance, a tendancy towards paradoxic hyper aggregability is seen.

Platelet response to aggregatory agents in patients in whom Aspirin has been withdrawn for > 10 days.

Patients waiting for CABG in whom Aspirin is withdrawn for > 10 days were evaluated-Group III. Compared againt controls. Quantitative platelet parameters were not statistically significant between 2 groups P > 0.05

Aggregometry with collagen and ADP
Slope and amplitude of collagen aggregometric curves
Collagen 5 µgm
Amplitude : group II Control : 65.114 + 19.81
III withdrawn : 64.77 + 15.16 P = 0.946

Slope	group II	73.68 + 23.78	
	III	83.59 + 21.73	P= 0.120
Collagen 10μ gm			
Amplitude	group II	67.57 + 20.60	
	III	67.04 + 04	P = 0.917
Slope :	II	76.51 + 24.87	P = 0.224
	III	84.18 + 19.37	
Analysis of ADP aggregation curves			
ADP 10μ mol			
Amplitude	group II	38.97 + 16.81	P = 0.125
	III	45.63 + 13.80	
Slope group	II	55.25 + 17.82	P= 0.002
	III	70.63 + 17.59	
ADP 20 mol			
Amplitude	group II	45.05 + 21.33	
	III	50.72 + 16.75	P= 0.295
Scope group	II	61.08 + 20.32	P= 0.109
	III	70.22 + 21.07	

Platelet aggregation was not inhibited compared to controls in patients who had stopped Aspirin for >10days indicating almost complete recovery of platelet function.

Comparison of aggregation tests between treatment group and drug withdrawn group (III)

Quantitative analysis of platelet function were similar (Table)

Aggregomety : with ADP and collagen as agonists

ADP aggregation curves			
ADP 10 μmol			
Group	I:	38.00 ± 15.64	P < 0.05
	III:	45.63 ± 13.80	

Slope		I :55.25 ± 17.82	P < 0.05
		III :70.63 ± 17.59	
ADP 20 µmol			
Amplitude		I: 45.05 ± 21.33	P= 0.193
		III :50.72 ± 16.75	
Slope		I 61 + 0.08 ± 20.32	P = 0.043
		III 70.22 ± 21.07	
Collagen aggregation curves analysis			
Collagen 5 µgm			
Ampitude		I 37.75 ± 19.94	P < 0.001
		III 64.77 ± 15.51	
Slope		I 35.53 ± 24.91	P< 0.001
		III 83.59 ± 21.73	
Collagen 10 µgm			
Amplitude		I 44.87 ± 20.60	P< 0.05
		III 67.04 ± 14.36	
Slope	I	39.21 ± 24.11	P < 0.001
		III 84.18 ± 19.37	

Aggregation parameter showed significantly reduced platelet aggregation compared to drug withdrawn groups.

Summary of results of aggregation studies

Group I

Platelet aggregation in patients on aspirin was significantly lower compared to control and drug withdrawn groups. $p < 0.05$

Group II

Platelet aggregation in drug withdrawn group is not significantly different from control group.

DISCUSSION

Present study assessed the biological effects of aspirin as measured by inhibition of platelet aggregation in patients taking aspirin for prevention of vascular diseases. 2 aggregatory stimuli were used (collagen, ADP) to determine the profile of ASA on platelet reactivity Measurement of blood levels of ASA is possible but not routinely done because of rapid metabolism of these agents, while plama Thromboxane can be measured, basal levels are generally very low and preclude detecting decrease in plama TX B2 as an index of ASA effect in reducing the platelet activity.[17] Study by Tohgi et al[18] measured serum thanboxane generated during the clotting of un anti coagulated blood in vitro and shows that serum TXB2 generation decreased significantly after 40 mg ASA / day and decreased further with increasing dose of ASA. Present study with lumi aggregometry did not show any better inhibition of platelet activities which doubling the dose of asprin, but eventhough not significant statistically, a tendency for hyper aggregability which requires further evaluation. Grotemeyer et al identified patients with higher than usual platelet aggregation 12 hrs after ingestion of 500 ASA and called such pts. secondary non responders.[7]

Present study shows approximately 50% inhibition of platelet aggregation induced by collagen and 20% inhibition of ADP induced aggregation suggesting aspirin is effective as an anti platelet agent, even though full inhibition is not found. There were no resistance to the given dose of aspirin in test group.

Quantitative analysis of platelet were almost similar in all the groups. Eventhough platelet distribution width can be used as an indicator of spontaneous micro aggregation. It was not prolonged either in test group or control groups.

In a pilot study, of patients taking ASA for prevention of recurrent stroke, Helgason et. al detected that different subjects required different ASA dosages to achieve inhibition of platelet aggregation[8].

Establishment of appropriate conditions for platelet aggregation tests:

There is no consistent correlation between bleeding time or thromboxare B2 levels and platelet aggregability. thromboxane B2 determination is not a direct measurement of platelet aggregation[18,19]. Maximum platelet aggregability in response to ADP and collagen is a practical test. It appears that measurement of platelet function in vitro allows one to monitor patients for presumptive evidence of desired asprin/Ticlopidine.

Physiologically collagen is also important in platelet adhesion, stage before aggregation. Aggregation is induced by the exposure of collagen lying beneath vascular endothelial cells and collagen is a factor in arterial thrombosis[20,21].

The working diagnostic threshold for aspirin was defined as a reduction of maxium aggregation rate < 60% at a final collagen. Concentration of 2μgm/l. In present study at a concentration of 5μgm/l, 50% inhibition of aggregation was noticed.

Concern regarding the use of different preparation of aspirin has been raised. Ali et al studied the plasma salyalate and platelet cyclooxygenase activity following enteric coated aspirin and found that comparable inhibition of cyclo oxygenase activity was noticed compared to non enteric coated tablets. The effect was delayed reflecting the delayed appearance of ASA in plasma.[23]

While ASA inhibit cyclooxygenase dependent pathways of platelet activation, it doesnot inhibit cyclooxygenase independent pathways (like thrombin induced platelet activation). In addition, ASA does not affect the binding of ligands other than fibrinogen to platelet glycoproteins IIb/IIIa. (for eg. von Willebrand factor). The latter mechanism of platelet activation may be induced by shear force alone. Under conditions of severe degrees of stenosis and lower shear forces, but injured endothelium, ASA would be expected to prevent platelet

initiated thrombosis. Shear stress related activation of platelets is mediated mainly by ADP where thienopyridines may be of better use. ASA may be ineffective in shear stress induced platelet activation.

Previous studies used much lower concentration of collagen 2μgm and ADP - 2μmol for detection of aspirin resistance. Present study utilised higher concentrations of aggregatory agonist and demonstrated adequate levels of inhibition. Present study clearly demonstrates that standard dose of aspirin produce significant inhibition of platelet activation. This underscores the beneficial effect of aspirin in vascular disease. There is no significant benefit upon increasing the dose of aspirin to double the dose, but this is a non significant trend towards hyper aggregability.

Limitations of study

Only 2 agents were used to evaluate the platelet function. Epinephrine and arachlidonic acid if used would have added to the outcome of the study. Number of patients, compared to other studies is small and only 11 patients could be studied with 2 doses of the drug.

SUMMARY & CONCLUSIONS

- Patients with CAD, on standard dose162.5 mg of aspirin resulted in approx 50% inhibition of platelet aggregation in response to collagen & 20% tom30% inhibition of ADP induced aggregation

- No resistance to action of aspirin noticed in the population studied

- Withdrawal of ASA > 10 days cause return of platelet function to baseline control levels

- Doubling the dose of ASA has little effecton platelet inhibition rather a non significant trend towards increased aggregation is detected which needs further evaluation.

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