P41

~~LIST OF PROCEDURES DONE~~

PROJECT REPORT

TITLE OF THE PROJECT:

A STUDY OF PLATLET AGGREGATION IN PATIENTS WITH VASCULAR HEADACHE (MIGRAINE) ASSOCIATED WITH CONGENITAL HEART DISEASE.

NAME..... DR. LEKHA BHASKARAN

PROGRAMME:..... DM. NEUROLOGY

MONTH & YEAR OF SUBMISSION:..... NOVEMBER 1988

forwarded

12.11.88

Note:— (i) In the case compilation of procedures done, the contents and the subsequent pages should be made into different sections (a) Procedures done (b) Procedures assisted (c) Procedures participated (d) Procedures attended/participated etc in Other Centres. Each section should be preceded by a leaf carrying the name of the section that is succeeding.

(ii) The Contents page will carry into. as per model given under

PROCEDURES DONE

Closed Mitral valvotomy.....124 (say)
Patent ductus arteriosus-ligation.....10
Atrial septal defects.....20
.....
.....

PROCEDURES ASSISTED

Closed Mitral valvotomy.....100 (say)
.....

(iii) In the subsequent pages details of each procedure done/assisted should be given in the format given below:—

Heading: **Closed mitral valvotomy**

Date	Name of the patient	Age	Sex	Patient No.

(iv) In the case of Project Report in the page immediately following the Certificate page the under-mentioned details should be given:—

(a) Title
(b) Duration
(c) Aim and scope
(d) 50 word summary of work done

CERTIFICATE

I, Dr. LEKHA BHASKARAN hereby declare that I have actually ~~performed all the procedures listed~~/carried out the project under report.

Signature: Lekha Mohan

Place: TRIVANDRUM

Name in capital letters: LEKHA BHASKARAN

Date: 10.11.88

CERTIFICATE

Certified that the work done in the project entitled " A STUDY OF PLATELET AGGREGATION IN PATIENTS WITH VASCULAR HEADACHE (MIGRAINE), ASSOCIATED WITH CONGENITAL HEART DISEASE " has been carried out by Dr. Lekha Bhaskaran herself under our guidance and supervision.

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A STUDY OF PLATELET AGGREGATION IN PATIENTS WITH VASCULAR HEADACHE (MIGRAINE) ASSOCIATED WITH CONGENITAL HEART DISEASE

By

LEKHA BHASKARAN

Under the guidance of:

Dr. P.K. MOHAN

Dr. M.P. JAMALUDDIN

***

1. Title of the Project: A study of platelet aggregation in patients with vascular headache (Migraine) associated with congenital heart disease.

2. Duration: Four months.

3. Aim and scope: The present study is undertaken in order to find out whether there is platelet hyperaggregation in patients of congenital heart disease having migraine; because our clinical experience suggests that the incidence of classical migraine is higher in patients of congenital heart disease, as compared to acquired cardiac disorders.

4. Summary: Patients of congenital heart disease (CHD) having migraine are found to have platelet hyperaggregation as compared to patients of congenital heart disease without migraine. This is much more marked in cyanotic CHD group. On the other hand, patients of CHD without migraine are found to have hypoaggregation of platelets as compared to control subjects.

CONTENTS

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ACKNOWLEDGEMENT

I am grateful to Dr.P.K.Mohan and Dr.Jamaluddin for their help and able guidance, in conducting this study. I am thankful to the department members in Pathology for their co-operation.

I am grateful to Dr.Balakrishnan, Professor of Cardiology for granting me permission to study patients admitted under his department.

I am grateful to Professor Valiathan for his kind permission to carry out this study.

Lastly, I thank all patients and volunteers who took part in this study.

Lekha Bhaskaran

Dated: 10.11.88. (LEKHA BHASKARAN)

INTRODUCTION

Association of migraine with congenital heart disease has not been studied in detail earlier. The present study is a continuation of an earlier study done from this department, on the above subject.

The basis of this study was the observation in our centre, that a large number of patients suffering from congenital heart disease (especially cyanotic heart disease) had migraine, especially classical migraine.

That, these patients, in addition to polycythaemia and other haematological disorders, also have platelet dysfunction and hence migraine, is the hypothesis proposed and tested in the present study.

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REVIEW OF LITERATURE

Migraine:

The term migraine is derived from French-Megrim, which in turn originated from the Latin-"Hemicranie" and its corrupted forms 'Hemigraniae' and 'Migranea'.

The exact pathogenisis of migraine has not been Clearly elucidated, since its first discription by Arataeus of Capadeeia in the first century. The various theories proposed are Vascular[1] neural[2], and blood disorder, the last refering to platelet abnormalities[3].

There is evidence to suggest platelet hyperaggregation (5,6) and platelet release reactions during an acute attack of migraine(4,7). Serotonin levels in the urine increase during an acute attack of migraine(8), accompanied by low levels in plasma, due to loss of the amines from the platelets(7,8). Betathromboglobulin- a platelet release reaction product is also increased during headache.

Platelet hyperaggregability has been shown in various studies comparing migraneurs with controls(5, 9-11). Hyperaggregability also corresponds well with the severity of migraine(3). However, studies by Couch and Hasseneri(11) could not support these findings - that hyperaggregability states correlated with severity of headache or with migraine associated with neurologic defict.

Migraine has been found to be frequent in patients with mitral valve prolapse syndrome with an incidence of 27.8% (Litman and Freedman[12]) as compared to 10% in general population.

Apart from Mitral valve prolapse, the association of migraine with other congenital heart diseases has not been established.

However, platelet functions have been studied in patients with congenital heart disease. Maurer et al(13) demonstrated for the first time, a defect in platelet aggregation in vitro in 37.8% of patients of congenital heart disease of the cynotic variety.

This platelet hypoaggregability was correctable by reducing the red cell volume, indicating that the defect in platelet function may be partly related to the accompanying polycythaemia.

Turitte and Keiss(14) studied the influence of R.B.C. concentration on platelet adhesion and thrombosis formation in vitro and found that high concentration increased platelet adhesiveness and low concentrations decreased their adhesiveness.

Livio et al(15) observed that prolonged bleeding time in uraemic patients could be corrected by red cell transfusion alone, suggesting that red blood cells have an important role in platelet function and haemostasis.

****

AIMS AND OBJECT

To study platelet aggregation rate by the Spectrophotometric method of Jamaluddin and Krishnan in:

(a). Congenital heart disease patients, both cyanotic and acyanotic.

(b). In Rheumatic and other acquired heart disease patients.

(c). Age and sex matched healthy subjects as controls.

Patients with heart disease, with and without migraine were included in this study.

****

MATERIAL AND METHODS

Only patients with confirmed congenital and rheumatic heart disease attending the Cardiology services of this centre were included in the study. They were divided into two groups. (i) with migraine (ii) without migraine.

The controls were healthy subjects without headache and were matched to the patients for sex and age ± 5 years. Diagnosis of migraine was based on the criteria of Lance(16)- headache predominantly unilateral and paroxysmal, associated with anorexia, nausea and vomiting. These with episodic headache, proceeded by visual disturbance or other focal neurological symptoms for 10-60 minutes and disappearing after development of headache were considered to be having classic migraine. Episodic headache with nausea or vomiting, without preceding visual disturbance or neurologic symptoms, was diagnosed as common migraine. Only these patients who had classical migraine according to the above criteria were included in the present study.

None of the patients were taking drugs known to interfere with platelet aggregation for at least one week prior to the study. Patients with hypertension, history of dyscollagenosis, and active allergy were excluded. Females were not

taking oral contraceptives. None of the patients were in cardiac failure. There was no history of headache in the proceeding two days prior to blood collection or history of aggravation of underlying cardiac problem in any of the patients.

Patients were kept fasting for 6 to 8 hours prior to blood collection. All samples were collected before 8 A.M. and analysed within two hours of collection. Alongside, Haemoglobin, packed cell volume and platelet count were done on the same day. Bleeding time was not measured because of lack of a template for precision.

Blood was drawn by means of a siliconised glass syringe and a 20 gauge needle and collected in plastic tubes and mixed with anticoagulants - Acid citrate Dextrose(A.C.D.) in ratio of one part of anticoagulant to 9 parts of blood. Samples were centrifuged at 500 r.p.m. for 30 minutes at room temperature, and platelet rich plasma(P.R.P) was pippeted out. Sufficient P.R.P. could not be obtained in patients with high haematocrit, hence normal saline was used as a diluent for P.R.P.

Platelet aggregation tests were done using Shimadzu UV-240 microprocessor controlled spectrophotometer, equipped with thermostat cuvette holders. All tests were done at 34 $\pm$ 1^{o}C.

P.R.P. was diluted with necessary amount of normal saline to give an absorbance of $\sim$ 0.6 at 540 nm. against air in a pair of rectangular silconised glass cuvettes with a path length of

10 nm. and placed in the sample and reference positions. The instrument was set to read zero at 540 nm.

Only Adenosine diphosphate (A.D.P.-Sigma) was used as an agonist, in the concentration of 2 and 10 micromolar. ADP was added by means of a micropippete to the sample cuvette and this was quickly inverted on a parafilm for mixing and placed back in the spectrophotometer in the sample position. Prior to this the reference cuvette was also treated in the same way by shaking to balance out any optical effects of relaxation of discoid platelets after shaking. The change in absorbance (turbidity) were recorded as a function of time, after appropriate choice of scale. Expansion and chart speed which were kept at - 0.06 to 0.06 and 10 nm./cm, respectively. A tangent was drawn to the steady linear downward pen deflection, by visual inspection and the slope expressed as Δ A/min., which was taken as the initial rate of aggregation.

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RESULTS

The distribution of case material and the nature of heart diseases are shown in tables 1 and 2 respectively.

Patients were divided into three groups:

(i) Controls - healthy volunteers
(ii) Congenital heart diseases
(iii) Rheumatic heart disease

TABLE I - CASE MATERIAL

GROUP			TOTAL NO.
I. Controls			
(i) healthy volunteers with headache	10	18	
(ii) without headache	8		
II. Congenital Heart Disease			
A. with migraine			
(i) cyanotic	10	20	
(ii) Acyanotic	10		
B. without migraine			
(i) cyanotic	10	19	
(ii) Acyanotic	9		
III. Rheumatic Heart Disease			
with migraine	10	20	
without migraine	10		
TOTAL		77	

NATURE OF HEART DISEASE - TABLE 2

	with migraine (no:)	without migraine (no:)
Cyanotic C.H.D.		
Tetrology of Fallot	9	10
Single atrium	1	
Acyanotic Heart Disease		
Atrial Septal Defect	2	5
Ventricular Septal Defect	6	2
Patent Ductus Arteriosus	-	1
Pulmonary Stenosis	1	1
Bicuspid Valve	1	-
Rheumatic Heart Disease		
Mitral stenosis	4	1
Mitral stenosis with mitral incompetence	2	2
Mitral incompetence	1	2
Mitral incompetence with Tricuspid regurgitation	1	1
Mitral stenosis with incompetence with Tricuspid regurgitation	-	1
Aortic stenosis with Aertic incompetence	1	1
Aortic stenosis	1	-
Total	30	29

Age and Sex distribution of patients is shown in Table 3

TABLE 3 : AGE AND SEX DISTRIBUTION

AGE GROUP	GROUP I A.	B	IIA	IIB	III	TOTAL NO.	M : F
0 - 10	-	-	5	6	1	12	8 : 4
11 - 20	5	4	12	9	7	37	23 : 14
21 - 30	1	3	3	4	6	17	4 : 13
31 - 40	1	2	0	0	4	7	4 : 3
41 - 50	1	1	0	0	2	4	1 : 2
51 - 60	0	1	0	0	0	1	0 : 1
TOTAL							40 : 37

TABLE 4 : MEAN PLATELET AGGREGATION RATES (MEAN ± S.A.)

	WITH HEADACHE ADP Concentration		WITHOUT HEADACHE ADP Concentration	
	2 μM	5 μM	2 μM	5 μM
Healthy Control	0.0180 ±0.006	0.0228 ±0.0094	0.02096 ±0.0094	0.02592 ±0.0010
R.H.D.	0.02144 ±0.001	0.02818 ±0.002	0.01662 ±0.0012	0.02390 ±0.009
Cyanotic Heart Disease	0.03544 ±0.001	0.02424 ±0.001	0.1488 ±0.007	0.0156 ±0.009
Acyanotic H.D.	0.02216 ±0.001	0.02616 ±0.001	0.01960 ±0.001	0.02606 ±0.001
C.D.H. (combined)	0.0288	0.0252	0.01724	0.02080

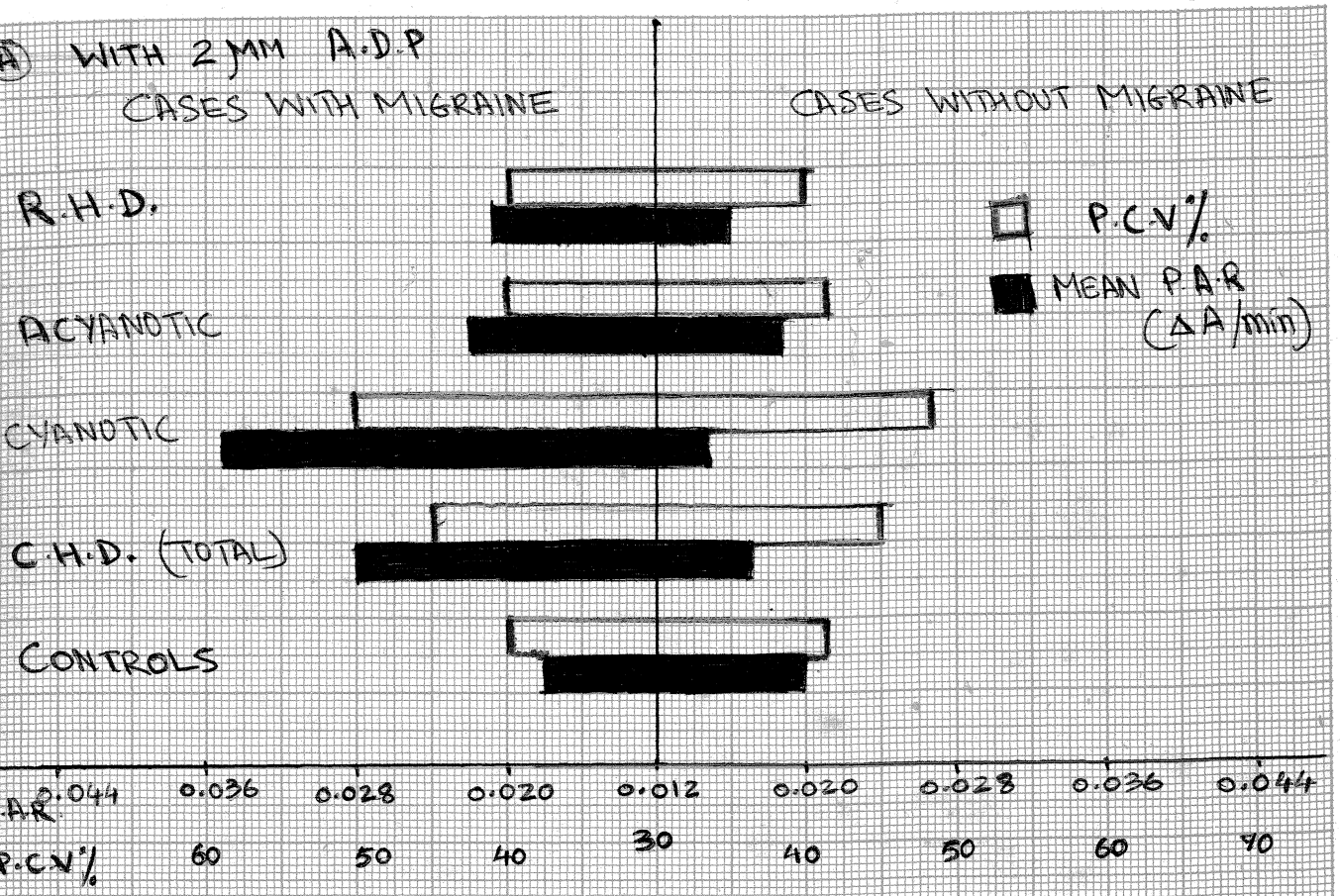
From table 4 it is clear that

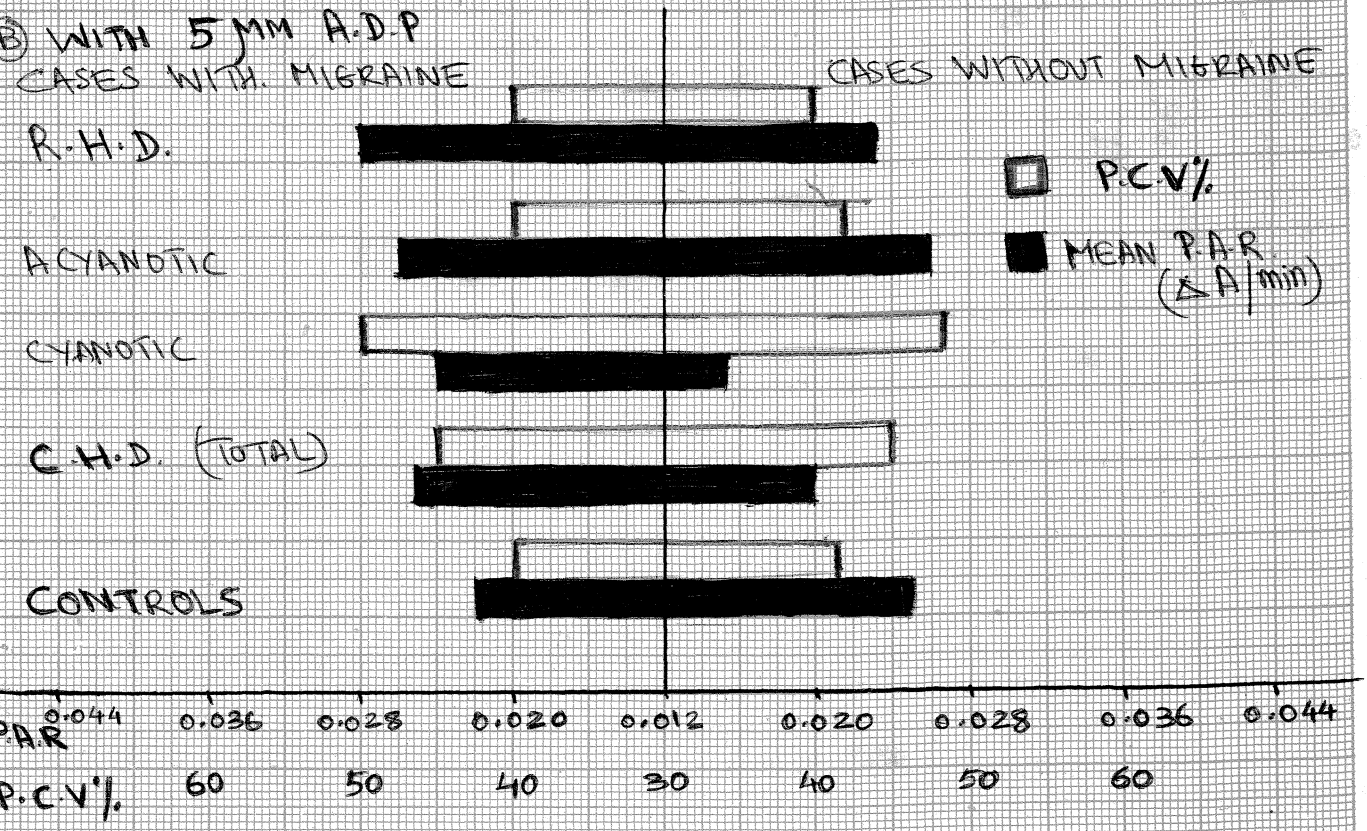
1. Mean P.A.R. of the C.H.D. group as a whole (without headache) is low as compared with controls and acquired heart disease, especially for the group with cyanotic heart disease.

2. The mean P.A.R. in C.H.D. as a whole with headache, is high as compared with those without headache. This difference is seen both with 2 μ and 5 μ ADP.

3. Patients with cyanotic heart disease with migraine show maximum aggregability as compared to Noncyanotic and acquired heart disease patients.

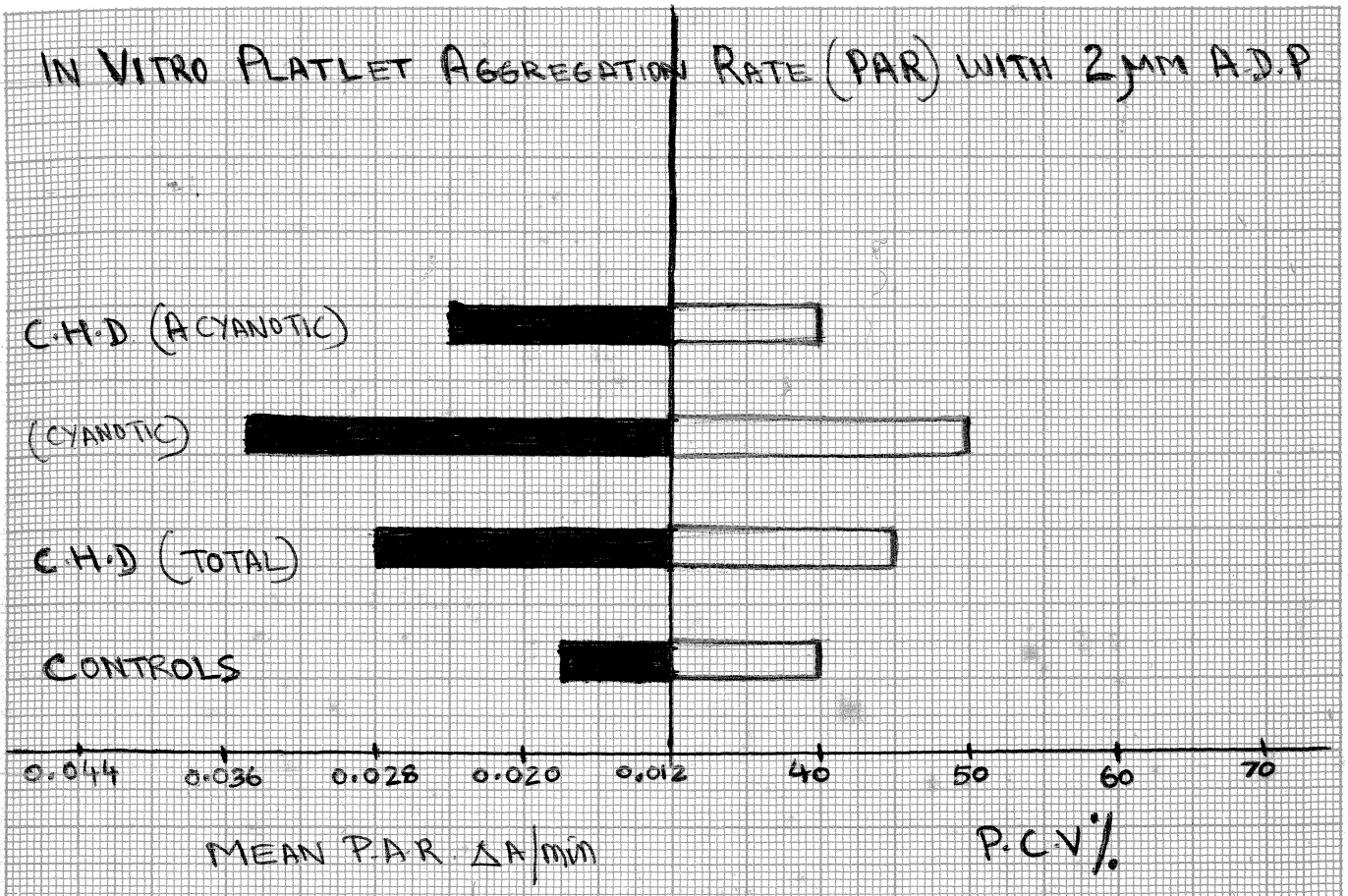
4. Healthy volunteers with migraine showed lower aggregation rates of platelets as compared to controls.

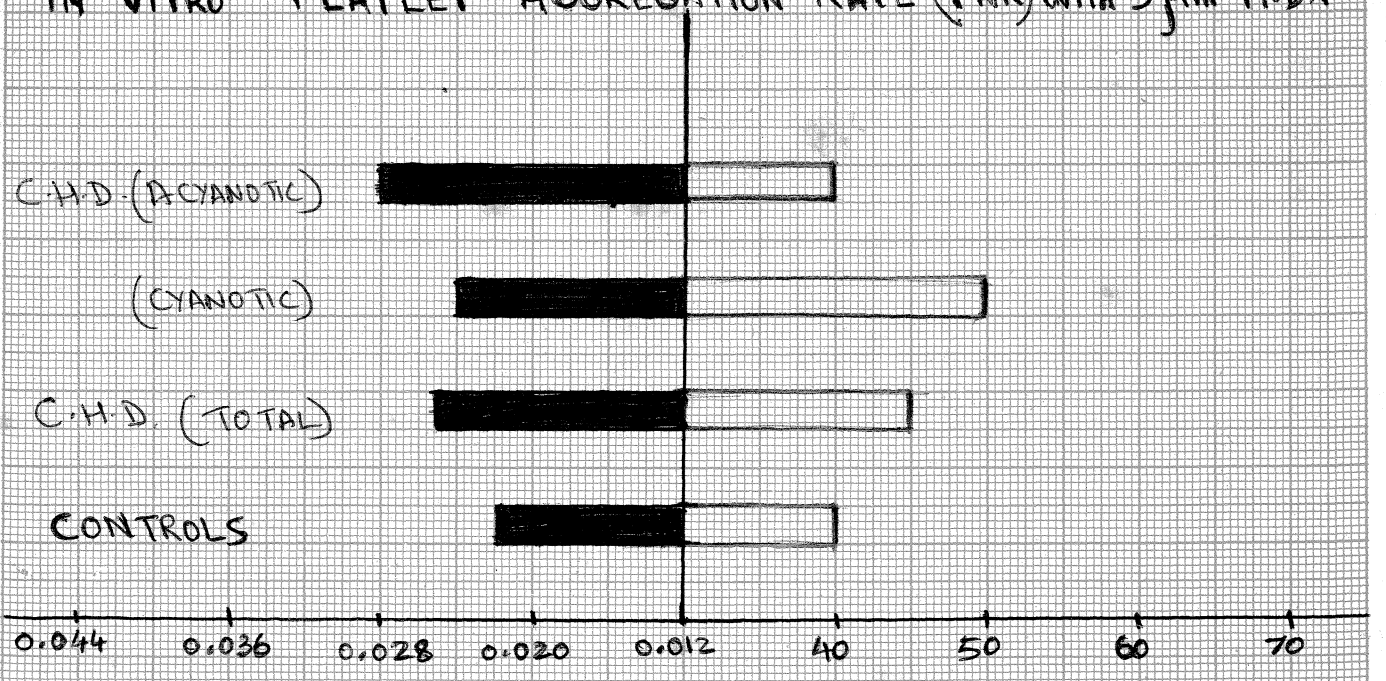




SHOWING MEAN PLATLET AGGREGATION AND P.C.V WITH AND WITHOUT MIGRAINE

FIG. 1





MEAN PLATELET AGGREGATION RATES AND P.C.V. IN VARIOUS GROUPS

FIG. 2

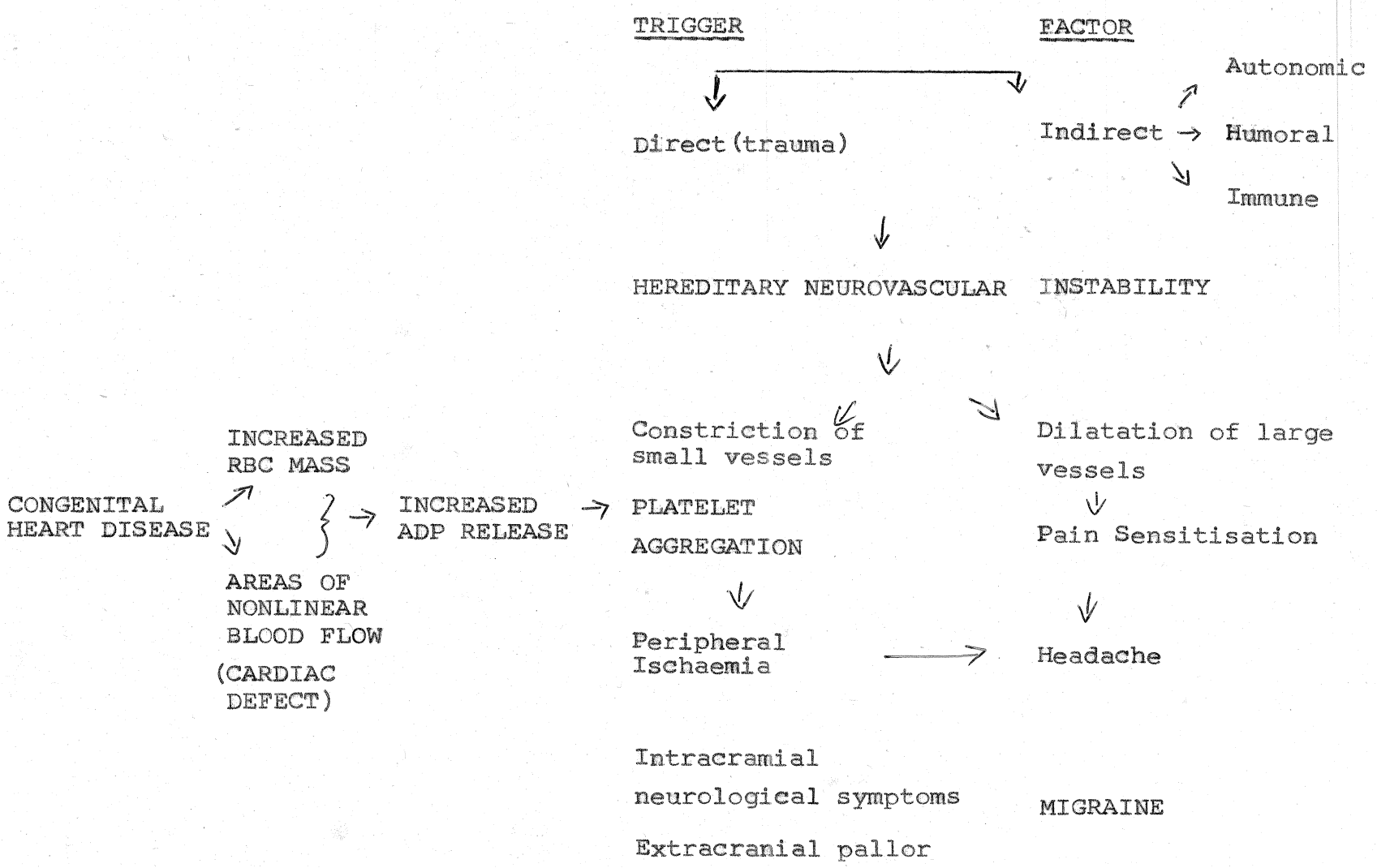


Fig. 3 POSTULATED HYPOTHESIS FOR MIGRAINE WITH CONGENITAL HEART DISEASE

DISCUSSION

The present study of platelet aggregation was undertaken based on (i) Our clinical observation that the incidence of classical migraine is higher in patients of congenital heart disease(CDH), especially cyanotic CDH., although this needs statistical evaluation, and

(ii) The reported hyperaggregability of platelets in migraine(12-14). It has been reported in literature that red blood cells may release ADP in vivo, in areas of nonlinear blood flow, as in atherosclerotic vessels(18),and this ADP released from red blood cells can produce platelet aggregation(19). It is well known that patients with cyanotic Congenital heart disease frequently have increased haematocrit(20). They also have various defects in the heart which may form areas of non-linear blood flow. Could these two factors bring about an increased release of ADP in vivo from red blood cells, leading to increased platelet aggregation and thereby result in migraine? This proposed hypothesis is diagramatically shown in figure(3).

The method used (17) in the present study for platelet aggregation in vitro was different from standard turbidometric technique(21) and had not earlier been studied in humans. Results in controls showed the reproducibility of this new

method. Increasing ADP concentration from 2 to 5 micromolar concentration resulted in a mild increase in platelet aggregation in controls.

Our findings cannot be compared with those in the literature because:

(i) We have not studied turbidometric method for comparison in the same subjects

(ii) Our method measures only the initial rate of platelet aggregation, whereas other tests measure the later part of the aggregation process(17).

Patients with congenital heart disease(CHD)had a slightly lower mean platelet aggregation rate PAR as compared to that of controls. However, the mean PAR in patients with cyanotic CHD was significantly lower, which was not so in acyanotic heart disease. This observation is consistent with earlier reports of platelet aggregation in cyanotic CHD(1-3). This platelet hypoaggregation was postulated to be due to a qualitative dysfunction(13). As we have used saline to dilute platelet rich plasma PRP, it can be argued that this might have resulted in alteration in some factor in the plasma. But an earlier study by Maurer et al(13) have found this possibility unlikely, because the platelet dysfunction noted un their study was not

correctable with normal donor plasma. As once of our patients were taking aspirin or any other drug known to affect platelet function(21), this abnormality appears to be associated with cyanotic CHD itself. Maurer et al(13) also found higher concentration of ADP(20) to improve the aggregation in some patients. This however was not tested in the present study.

C.H.D. - MIGRAINE - PLATELET AGGREGATION:

The mean platelet aggregation rate in C.H.D., was found to be higher in these with migraine as compared to that of C.H.D. without migraine, (fig. 1). The mean PAR in cyanotic C.H.D. patients with migraine was much higher than that of those without migraine, and the control groups.

In the acyanotic CHD group without migraine, the mean PAR was similar to that in control group, but patients with migraine had higher mean PAR than controls. The patients of Rheumatic heart disease with migraine were also found to have increased mean PAR as compared to those without, and controls.

There was no consistent relationship between PAR and packed cell volume as seen in the earlier study from this centre. (This study had shown that in cyanotic CHD without migraine there was low PAR with increased PCV). (See figure 2).

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SUMMARY AND CONCLUSION

1. Patients with congenital heart disease have hypoaggregability of platelets as compared to controls.
2. Patients with congenital cyanotic heart disease without headache are found to have hypoaggregability of platelets as compared to patients with acyanotic heart disease without headache. The same does not hold good for the same category of patients with headache.
3. Patients with congenital heart disease with migraine are found to have hyperaggregation of platelets as compared to patients of congenital heart disease without migraine.
4. The above observation is much more striking in patients of cyanotic congenital heart disease.
5. Patients with acyanotic CHD having migraine are found to have hyperaggregation of platelets as compared to those without migraine and also control subjects.

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