Elevated fibrinogen and lowered homocysteine-vitamin determinants and their association with left atrial thrombus in patients with rheumatic mitral stenosis

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ABSTRACT

Mitral stenosis (MS) causes stagnation of blood flow, leading to thrombus formation in the left atrium (LA), which may lead to systemic thrombo-embolic complications and stroke. We compared the alterations in echocardiographic and procoagulant parameters in patients with severe rheumatic MS with and without LA thrombus. The study was a cross-sectional study of patients with rheumatic MS, being evaluated for percutaneous mitral commisurotomy. Group 1 patients comprised of patients with rheumatic MS with LA thrombus (n=35) and Group 2 patients had rheumatic MS without LA thrombus (n=45). Platelet aggregability, fibrinogen, homocysteine, vitamin B_{12} and folate; mitral valve area (MVA), mean mitral gradient and pulmonary artery pressure (PAP) were assessed in all study subjects. Significant increase in fibrinogen, homocysteine and platelet aggregation and fall in homocysteine-associated determinants were seen in Group 1, as compared with Group 2. Raised fibrinogen, lowered homocysteine-vitamin determinants and lowered mitral valve area were associated independently, with presence of LA thrombus in rheumatic MS. In this study, fibrinogen, vitamin B_{12} and folate were independently associated with the occurrence of thrombus in patients with rheumatic MS. Hence, our results suggest that increase in procoagulant mechanisms contribute to increased risk of thrombosis in the left atrium in patients with rheumatic MS.

KEY WORDS: Rheumatic mitral stenosis.
Left atrial thrombus.
Platelet aggregability.
Fibrinogen.
Homocysteine.
Vitamin B12.
Folate.

Introduction

In India, rheumatic fever is endemic and remains one of the major causes of cardiovascular disease, accounting for nearly 25–45% of the acquired heart disease.[1,2] The incidence of systemic embolism is greater in rheumatic mitral valve disease than in any other common form of valvular heart disease.[3] It has been observed particularly in the left atrium (LA) and left atrial appendage in low flow states were high risk for development of thrombus. The extent of blood stasis within the appendage and its contractile function may be important determinants of thrombo-embolic risk.[4] However, this does not explain all the cases of LA thrombus in MS. The reason for the thrombi to develop in only some patients but not in others is unknown.

Homocysteine is a sulphur amino acid and a normal intermediate in methionine metabolism. It facilitates oxidative arterial injury, damages the vascular matrix, and induces vascular smooth muscle proliferation. Interference of homocysteine with the coagulation system creates a pro-thrombotic milieu promoting formation of LA thrombus in MS.[5] Fibrinogen plays a major role in blood clotting, platelet aggregation, fibrinolysis, cellular and matrix interactions, the inflammatory response, wound healing, and neoplasia. Fibrinogen has been implicated as a molecular bridge between activated cells during platelet aggregation.[6] Platelet activation occurs in the peripheral blood of patients with MS. Activated platelets release fibrinogen from its α-granules.[7] Released fibrinogen as well as von Willebrand factor (vWF) binds to receptors on one platelet and cross links to the other platelet by binding on the receptors on the latter thereby causing platelet aggregation and hence thrombosis.

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Hence, we undertook to study the alterations in the echocardiographic (MVA, mean transmitral gradient, pulmonary artery pressure) and procoagulant (homocysteine, its vitamin determinants, fibrinogen and platelet aggregation) parameters in patients with severe rheumatic MS with and without LA thrombus and to study their predictive power to detect the presence of LA thrombus in patients with rheumatic MS.

Table 1. Comparison of study parameters between the groups.

Sl No.	Parameter	Group 1 MS with LAT (n=35)	Group 2 MS without LAT (n=45)	P value
1	Age (years)	40.8±7.0	38.5±8.6	0.200
2	Waist:hip ratio	0.86±0.05	0.86±0.04	0.882
3	BMI (kg/m^2)	20.4±4.0	20.4±4.3	0.969
4	Fibrinogen (g/dL)	3.6±0.8	2.9±0.8	<0.0001
5	Vitamin B_{12} (pg/mL)	326.0±98.6	404.1±188.8	0.029
6	Folate (ng/mL)	5.7±1.8	7.2±3.3	0.023
7	Homocysteine (μmol/L)	32.4±18.8	23.8±10.2	0.010
8	Platelet aggregation (ohms)	9.05±2.41	7.92±2.42	0.041
9	Mean MVA (cm^2)	0.92±0.11	1.00±0.11	0.001
10	Mean mitral gradient (mmHg)	17.7±4.8	17.2±4.1	0.567
11	AF	32 (91.4)	30 (66.6)	0.018*
12	PAP (mmHg)	73.6±19.6	68.4±16.8	0.208

*Fischer's exact test

MVA: mitral valve area; AF: atrial fibrillation; PAP: pulmonary artery pressure.

Materials and methods

This was a cross-sectional study involving 80 consecutive patients with symptomatic severe rheumatic MS (mitral valve area on routine transthoracic echocardiography [TEE] <1.2 cm^2), undergoing TEE evaluation at our hospital (a tertiary care centre in South India) for occurrence of LA thrombus, before percutaneous mitral commisurotomy, who satisfied the inclusion and exclusion criteria. The participants were divided into two groups based on presence (Group 1) or absence of thrombus (Group 2), by TEE evaluation. Of the 80 patients recruited in the study, 35 had thrombus and constituted the Group 1 and the rest were classified as Group 2.

Patients with clinical evidence of active rheumatic activity, mitral regurgitation, prior anticoagulation therapy (warfarin/heparin), history of known coagulation abnormalities, diabetes, hypertension, endocrine disorders, smoking, renal/liver impairment, infective endocarditis, neoplasia, connective tissue disease, deep vein thrombosis or pulmonary embolism were excluded from the study. None of the patients in the study population had stroke, cardiovascular or peripheral vascular disease. Written informed consent was taken from all study subjects, prior to recruitment. Ethical approval was taken from the Institute's Human Ethics Committee. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Detailed history was taken and clinical examination was performed in all study subjects. Echocardiographic parameters (MVA, mean mitral gradient and PAP) were assessed in all study subjects.

Procoagulant parameters were estimated in the 6 mL fasting blood sample drawn immediately, after the TEE in both the groups. During the same visit, anthropometric measurements (weight, height) were recorded. Body mass index (BMI) was calculated as per standard formula as the ratio of weight in kilograms and the square of the height in metres. Routine biochemical investigations like blood glucose, lipid profile and uric acid were estimated in all study subjects using standard methods using reagent kits from Agappe Diagnostics (Agappe Diagnostics, Kerala, India) and Erba Diagnostics (ERBA Diagnostics, Mannheim, Germany). All the above estimations were carried out in a well-calibrated automated clinical chemistry analyzer, RX Imola (Randox Laboratories, Crumlin, UK).

Assay of study parameters

Biochemical parameters were estimated in the 6 mL fasting blood sample (2 mL each collected in three different vials-EDTA vial, citrated vial and plain vial) drawn immediately, after the TEE in both the groups, after taking informed consent. Citrated blood was used for estimation of fibrinogen and platelet aggregability. Blood collected in EDTA vial was used for estimation of plasma homocysteine. Serum was used to measure the levels of vitamin B_{12} and folate. Plasma fibrinogen was estimated by quantitative immunoturbidimetric assay using kits from Tulip Diagnostics, Goa, India. Plasma homocysteine, serum vitamin B_{12} and folate levels were estimated by competitive immunoassay using direct chemiluminescence technology in the Advia Centaur CP Immunoassay system (Siemens, Healthcare Sector, Erlangen, Germany).

Platelet aggregation was estimated by impedance method, using Model 700-2 Chronolog aggregometer (West Park Road, Havertown, PA). Platelet aggregation studies were performed, half an hour after collection of sample. To avoid possible observer bias, blood samples were coded and blinded. Sampling procedures and platelet studies were performed by investigators unaware of the protocol.

Adenosine diphosphate (ADP; Sigma Chemical, St. Louis, MO, USA) was used as the aggregating agent. Platelet aggregation was stimulated with 10 μmol ADP. Platelet aggregation was expressed as the change in electrical impedance and expressed in ohms. Aggregation curves were recorded for 6 min and analysed using AGGRO/LINK8 control software (Chronolog, USA).

Statistical analysis

Statistical analysis was performed using IBM SPSS statistics (version 20) for Windows. Both descriptive and inferential

Table 2. Multivariate binary logistic regression analysis to determine independent factors associated with LAT

Sl No.	Parameter	Multivariate analysis Adjusted odds ratio	95% CI	
			Lower	Upper
1	Fibrinogen	1.013*	1.005	1.021
2	Vitamin B_{12}	0.994*	0.989	0.999
3	Folate	0.723*	0.558	0.936
4	Platelet aggregation	1.029	0.796	1.364
5	AF	4.998	0.937	26.638
6	MVA	0.000*	0.000	0.369

*Significant

statistics were used to analyse the data. Baseline characteristics of the patients with MS were analysed by descriptive statistics. Categorical data were described using percentages and frequencies and were compared by using Fischer's exact test. The normality of continuous data was assessed by Kolmogrov-Smirnov test. Normally distributed data were described by mean ± standard deviation and median was used for non-Gaussian data. Normally distributed continuous data were compared by Independent Student's *t*-test and Mann Whitney U test was used for non-Gaussian data. The association of clinical, biochemical and echocardiographic variables with the occurrence of LA thrombus was analysed using univariate analysis. The variables found significant in univariate analysis were considered for multivariate logistic regression analysis. Analysis was carried out at 5% level of significance and $P<0.05$ was considered as statistically significant.

Results

The demographic parameters (age, BMI and waist-hip ratio) in the study groups were comparable (Table 1).[8] Plasma fibrinogen and homocysteine were significantly elevated, while homocysteine vitamin determinants, vitamin B_{12} and folate were lowered, in Group 1, as compared with Group 2 (Table 1). Platelet aggregability (measured as impedance in ohms) also showed a significant rise in Group 1, as compared with Group 2 (Table 1). The rise of fibrinogen and lowering of homocysteine-vitamin determinants persisted, after adjustment for confounding variables.

A comparison of the echocardiographic variables between the groups showed that the mean mitral valve area was lower in LA thrombus group, than in the group without thrombus. Mean mitral gradient and pulmonary artery pressure was not significantly different among patients with LA thrombus, as compared with those without LA thrombus.[8] Atrial fibrillation (AF) was more frequent in patients with LA thrombus (Table 1).[8]

The biochemical parameters found significant in univariate analysis were considered for multivariate logistic regression analysis. It showed that fibrinogen, vitamin B_{12} and folate and MVA were independent factors associated with the occurrence of thrombus in patients with rheumatic MS in the present study (Table 2). Fibrinogen was the best independent predictor of left atrial thrombus, with the highest odds ratio (Adjusted OR=1.013, $P=0.003$) (Table 2).

Discussion

The present study investigated the echocardiographic and pro-coagulant parameters in patients with rheumatic MS and compared their alterations in patients with and without LA thrombus.

We found that plasma homocysteine, plasma fibrinogen and platelet aggregability were significantly elevated, whilst homocysteine vitamin determinants-vitamin B_{12} and folate levels were lowered in patients with rheumatic MS with LA thrombus, as compared with those without LA thrombus. This could explain the increased risk for thrombus formation in the left atrium in Group 1, as compared with Group 2.

These findings were in corroboration with a previous study which demonstrated hyperhomocysteinaemia in patients with LAT in stroke[9] and supports the evidence that high plasma homocysteine could contribute to the LA thrombus formation. Even though pathophysiological mechanisms by which hyperhomocysteinaemia is linked with thrombus formation is unclear, several studies have postulated that homocysteine may alter the thrombotic properties of the endothelium by inhibiting the expression of thrombomodulin, activating protein C, enhancing the activity of factors XII and V, and augmenting platelet adhesion to the endothelial cells.[9–12] Thus interference of homocysteine with the coagulation system creates a pro-thrombotic milieu thereby increasing thrombus formation.[6,12]

Platelet membrane phospholipids potentiate the intrinsic pathway of coagulation, which eventually forms thrombin from pro-thrombin by activated factor X. The constituents of the platelet release reactions play an important role in the formation of platelet thrombus. For example, platelet factor 4 seems to possess anti-heparin activity, and fibrinogen that is released could potentially contribute further to the formation of the thrombus.[13] Previous studies show that an interplay between vWF and fibrinogen is essential for the binding of vWF to the Gp 1 b receptor in the platelets, contributing to increased platelet adhesion, resulting in thrombus formation.[14]

Elevated plasma fibrinogen and lowered vitamin determinants were associated independently with thrombus in patients with MS. We found that the plasma fibrinogen was the best predictor for LA thrombus in patients with rheumatic MS. Contrary to our results, Karthikeyan *et al.*,[15] in their study involving 56 cases with left atrial thrombus and 55 without left atrial thrombus, reported that elevated

homocysteine levels and lowered folate and vitamin B_{12} were not associated with the presence of left atrial thrombus, on multivariate logistic regression for prediction of left atrial thrombus in rheumatic MS.

We observed that the incidence of AF was significantly higher in LA thrombus group, when compared to without LA thrombus group. However, the presence of AF was not a significant independent predictor of left atrial thrombus, on multivariate logistic regression analysis. Patients with MS and in particular those with atrial fibrillation (AF), are at increased risk of developing left atrial thrombus and the associated thromboembolic complications. Part of this risk may be attributed to the hypercoagulable state associated with AF, with abnormalities of haemostasis, platelets and endothelial dysfunction.[9]

Wang *et al*.[16] reported that coagulability is increased in the left atria of patients with MS. Phankingthongkum *et al*.[17] demonstrated an abnormal hypercoagulable state of the left atrium and systemic circulation, which contributes to left atrial thrombus formation in patients with mitral stenosis. In another study, the authors reported that platelet aggregation is higher in patients with rheumatic MS, than in healthy controls.[18] This adds to the increasing body of evidence of procoagulant mechanisms in left atrial thrombosis in patients with MS.

Li-Saw Hee *et al*. [19] reported that indexes of hypercoagulability in peripheral blood reflect levels in intracardiac blood in patients with atrial fibrillation secondary to MS. Hence, the relevance of our findings in this study increases manifold in patients with rheumatic MS. This suggests a putative role for procoagulant mechanisms in the pathogenesis of LA thrombus formation in rheumatic MS.

Manjunath *et al*.,[20] in their study involving a large cohort of 848 MS patients in sinus rhythm, detected 56 (6.6%) to have LA thrombus by TEE. On univariate analysis, there was a trend toward thrombus formation in individuals aged >44 years, LA inferosuperior dimension >6.9 cm, mean mitral gradient >18 mmHg and dense spontaneous echo contrast (SEC). On multivariate analysis none of the factors predicted clot formation.

We found that the mean mitral valve area was lower in the LA thrombus group, than in the group without thrombus, indicating that the extent of stenosis was higher in Group 1, contributing to the development of thrombus in rheumatic MS patients. Contrary to the study reported by Manjunath *et al*.,[20] we did not find any difference between the groups in the mean mitral gradient or the pulmonary artery pressure. This is in concordance with a previous study by Karthikeyan *et al*.[15]

Our study had a few limitations. We demonstrated that increased levels of fibrinogen and reduced levels of vitamin B_{12} and folate were significantly associated with the presence of LA thrombus in MS patients. However, it is unknown whether this result is due to cause or result. This was a cross-sectional study, which could not establish cause-effect relationship. Second, we could not ascertain the dietary practices of the study subjects, which could affect their vitamin status. A small sample size was another limitation in this study. We recruited 80 subjects with rheumatic MS in the study period and divided them into two groups, based on presence (Group 1, $n=35$) or absence of thrombus (Group 2, $n=45$). Hence, further studies are required involving a larger sample size to validate our results.

In conclusion, in the present research work, elevated fibrinogen and lowered homocysteine-vitamin determinants, folate and vitamin B_{12} were the independent predictors of LA thrombus in patients with rheumatic MS. The strength and nature of this association and the mechanisms involved need to be elucidated in future studies. Randomised clinical trials, using vitamin supplementation and/or anticoagulant therapy, would aid in devising more appropriate therapy in patients with rheumatic MS and prevent the formation of thrombus in the LA in rheumatic MS. □

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