Endothelin-1 Gene Polymorphisms in Severe Pulmonary Hypertension associated with Rheumatic Mitral Stenosis

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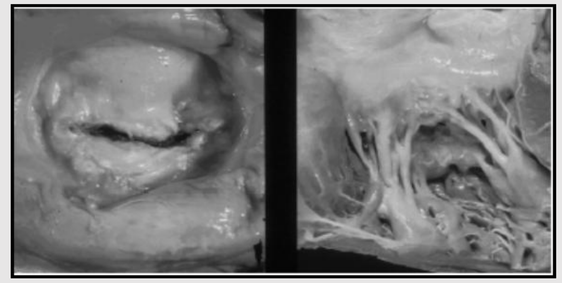


Introduction

❖ Rheumatic heart disease results from damage to heart valves caused by a single or recurrent episodes of rheumatic fever. It is endemic in developing countries including India.

❖ It has a worldwide prevalence of 33 million, resulting in about 3,50,000 deaths worldwide annually.

❖ Mitral valve is most commonly involved and pulmonary hypertension (WHO group II) is a common sequelae of rheumatic mitral valve disease.

❖ Endothelin-1 (EDN1) is a potent vasoconstrictor with mitogenic and angiogenic properties and has a crucial role in the pathophysiology of idiopathic pulmonary arterial hypertension (WHO group I).

❖ Studies have showed that genetics plays a major role in the pathogenesis of idiopathic pulmonary arterial hypertension (WHO group I) and formed the basis for drug therapy with Endothelin receptor antagonists.

❖ However, the influence of genetics on pulmonary hypertension associated with mitral valve disease (WHO group II) is yet to be determined.

❖ The genetic variants of EDN1 may be involved in the pathophysiology of pulmonary hypertension associated with rheumatic mitral stenosis, and hence we sought to study the role of endothelin-1 gene polymorphisms in its pathophysiology.

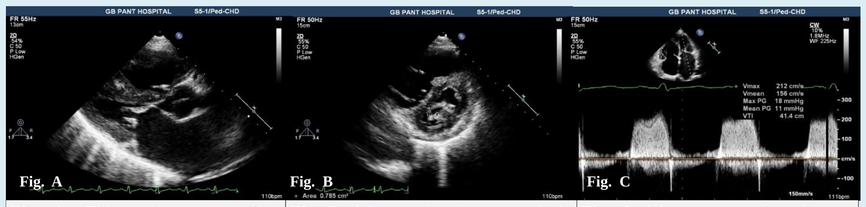


Fig. A: Thickened mitral leaflets with diastolic doming of anterior mitral leaflet with LA dilatation.

Fig. B: Mitral valve in short-axis in diastole showing fused medial and lateral commissures. MVA 0.78 cm^2.

Fig. C: Measurement of peak/mean diastolic transmitral gradient of 18/11 mm Hg by continuous wave Doppler.

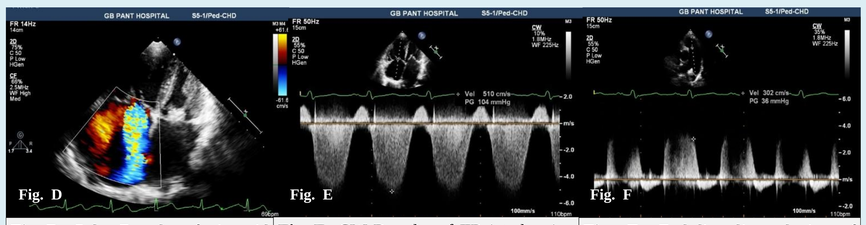


Fig. D: Color Doppler of tricuspid regurgitation (TR) jet in four chamber view. Note the dilated right atrium and right ventricle.

Fig. E: CW Doppler of TR jet showing peak velocity of 5.1 m/sec with calculated RVSP of 104 mm Hg plus right atrial pressure.

Fig. F: End-diastolic velocity of pulmonary regurgitation signal of 3 m/sec with calculated PAEDP of 36 mmHg plus right atrial pressure.

Echocardiography Examination Images of Patient with Rheumatic Mitral Stenosis and Severe Pulmonary Hypertension

Methods

❖ A total of 246 subjects were enrolled in the study comprising of 2 groups:

Group A
123 consecutive cases of Pulmonary Hypertension (PH) associated with isolated chronic rheumatic mitral stenosis

Group B
123 age and sex matched healthy controls

❖ All patients were enrolled over a period of 2 years from outpatient department of G.B. Pant Institute of Postgraduate Medical Education and Research, New Delhi.

❖ Demographics, history, clinical exam and detailed echocardiography exam done.

❖ Blood was collected for hemogram, anti-streptolysin O titre (ASO), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), endothelin levels (by ELISA) and anticoagulated blood sample for DNA analysis.

❖ DNA was extracted from peripheral blood leukocytes and genotyping was performed by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). Allelic and genotypic frequencies estimated in patient and control groups by appropriate statistical tests.

Gene	Polymorphism	Primer Sequence	Cycling Conditions	Product Size
Endothelin Gene	3A/4A	F:5'-GCTGCTTTTCTCCCCGTTAA-3' R:5'-CAAGCCACAAACAGCAGAGA-3'	I 95°C 10', D 95° 40", A 57.3°C 40", E 72°C 1', 39 cy, FE 72°C 10'	195bp
	Lys198Asn	F:5'-ATGATCCCAAGCTGAAAGGCTA-3' R:5'-CAGGGCTCTCCGTGGAGGCTAT-3'	I 93°C 3', D 93° 50", A 52°C 50", E 72°C 50", 34 cy, FE 72°C 10'	116bp

1. **3A/4A polymorphism** is insertion/deletion polymorphism located at exon 1 of endothelin-1 gene on chromosome 6 with three genotypes: Wild type (Homozygous Dominant): 3A/3A, Heterozygous: 3A/4A, and Mutant (Homozygous Recessive): 4A/4A.

2. **Lys198Asn polymorphism** is G to T transversion at location 5665 affecting 61st nucleotide of exon 5 of endothelin-1 gene on chromosome 6. The genotypes of this polymorphism are: Wild type (Homozygous Dominant): G/G (Lys/Lys), Heterozygous: G/T (Lys/Asn) and Mutant (Homozygous Recessive): T/T (Asn/Asn).

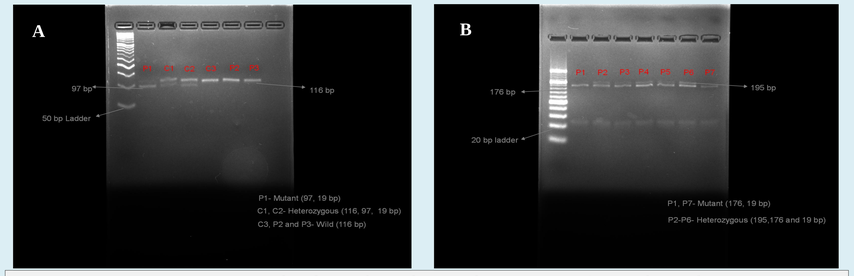


Image A: Restriction Fragment Length Polymorphism showing digestion-Lys198Asn polymorphism
Image B: Restriction Fragment Length Polymorphism-3A/4A polymorphism

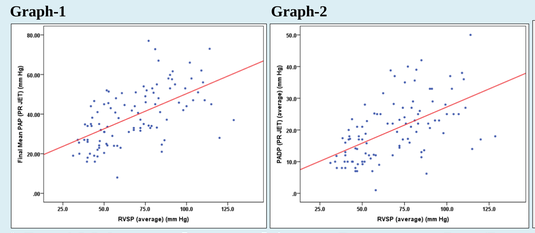
Results

❖ The mean age in Group A and Group B was 34.2±9.9 and 35.4±9.3 yrs (p=0.331) respectively, with female preponderance: 65% females-Group A, 61.8% Group B.

❖ In Group A, all patients had severe mitral stenosis with mean mitral valve area 0.81±0.18 cm^2. The mean diastolic trans mitral gradient was 11.74±4.71 mm Hg.

❖ The mean right ventricular systolic pressure was 70.26±24.11 mm Hg and mean pulmonary end diastolic pressure was 25.96±10.29 mm Hg suggesting severe pulmonary hypertension.

Graph-1 **Graph-2**



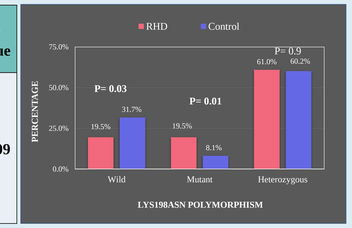
Graph-1: Correlation of RVSP (Right Ventricular Systolic Pressure) with mean pulmonary artery pressure

Graph-2: Correlation of RVSP (Right Ventricular Systolic Pressure) with pulmonary artery end-diastolic pressure

❖**Lys198Asn polymorphism**: Genotype Lys/Lys was present in 19.5% in Group A and 31.7% in Group B (p=0.04), genotype Lys/Asn was present in 61% in Group A and 60.2% in Group B (p=1), genotype Asn/Asn was present in 19.5% in Group A and 8.1% in Group B (p=0.02).

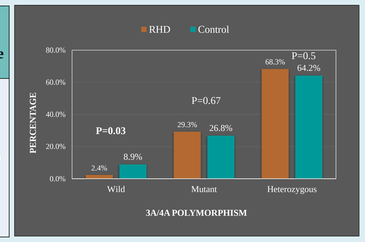
❖The frequency of Asn/Asn homozygous was significantly higher in Group A suggestive of association of Lys198Asn polymorphism with pulmonary hypertension associated with rheumatic mitral valve disease.

Endothelin-1 Gene Polymorphism	Category	RHD (Group A)		Control (Group B)		p-value
		Count	%	Count	%	
Lys198Asn Polymorphism	Wild (Lys/Lys)	24	19.5%	39	31.7%	0.009
	Mutant (Asn/Asn)	24	19.5%	10	8.1%	
	Heterozygous (Lys/Asn)	75	61.0%	74	60.2%	
	Total	123	100%	123	100%	



❖**3A/4A polymorphism**: Genotype 3A/3A was present in 2.4% in Group A and 8.9% in Group B (p=0.05), genotype 3A/4A was present in 68.3% in Group A and 64.2% in Group B (p=0.59), genotype 4A/4A was present in 29.3% in Group A and 26.8% in Group B (p=0.89) which shows no significant differences between both the groups.

Endothelin-1 gene polymorphism	Category	RHD (Group A)		Control (Group B)		p-value
		Count	%	Count	%	
3A/4A Polymorphism	Wild (3A/3A)	3	2.4%	11	8.9%	0.09
	Mutant (4A/4A)	36	29.3%	33	26.8%	
	Heterozygous (3A/4A)	84	68.3%	79	64.2%	
	Total	123	100%	123	100%	



❖ Endothelin levels were similar between the two groups.

Conclusions

Endothelin-1 gene polymorphisms appear to play a significant role in the pathophysiology of pulmonary hypertension associated with rheumatic mitral valve disease.

References

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