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Original Article

Gender differences in genotypic distribution of endothelin-1 gene and endothelin receptor A gene in pulmonary hypertension associated with rheumatic mitral valve disease

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ABSTRACT

Introduction: The female gender is a risk factor for idiopathic pulmonary arterial hypertension. However, it is unknown whether females with rheumatic mitral valve disease are more predisposed to develop pulmonary hypertension compared to males.
Aim: We aimed to investigate whether there was a difference in genotypic distribution of endothelin-1 (ET-1) and endothelin receptor A (ET_A) genes between female and male patients of pulmonary hypertension associated with rheumatic mitral valve disease (PH-MVD).
Methods: We compared prevalence of ET-1 gene (Lys198Asn) and ET_A gene (His323His) polymorphisms according to gender in 123 PH-MVD subjects and 123 healthy controls.
Results: The presence of mutant Asn/Asn and either mutant Asn/Asn or heterozygous Lys/Asn genotypes of Lys198Asn polymorphism when compared to Lys/Lys in females showed significant association with higher risk (odds ratio [OR] 4.5; p =0.007 and OR 2.39; p =0.02, respectively). The presence of heterozygous C/T and either mutant T/T or heterozygous C/T genotypes of His323His polymorphism when compared to wild C/C genotype in females showed a significant association with higher risk (OR 1.96; p =0.047 and OR 2.26; p =0.01, respectively). No significant difference was seen in genotypic frequencies in males between PH-MVD subjects and controls. Logistic regression analysis showed that mutant genotype Asn/Asn (p =0.007) and heterozygous genotype Lys/Asn of Lys198Asn polymorphism (p =0.018) were independent predictors of development of PH in females.
Conclusions: ET-1 and ET_A gene polymorphisms were more prevalent in females than males in PH-MVD signifying that females with rheumatic heart disease may be more susceptible to develop PH.

1. Introduction

Pulmonary hypertension (PH) is a chronic, progressive disease with guarded prognosis.[1] The female gender is a risk factor for idiopathic pulmonary arterial hypertension (PAH)[World Health Organization (WHO) group I PH] with greater proportion of female patients (56–86%) reported in several registries.[2] In the European Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA), the higher female to male ratio 2.3:1 was especially evident in younger patients (median age 54 years) compared to older patients (1.2:1, median age 75 years) highlighting the role of gender.[2] Also, connective tissue disease associated pulmonary hypertension has a 9:1 female to male ratio.[3] Further, females with liver disease have a higher risk of developing porto-pulmonary hypertension.[4] Multiple mechanisms might be responsible for the gender differences in the incidence of PH including sex hormones, immunologic triggers and genetic factors.[2]

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The genetic predisposition in concurrence with environmental factors probably plays a major role in the disease expression.

Many patients with chronic rheumatic mitral valve disease develop pulmonary hypertension (WHO group II PH) which may be progressive.[1,5] The incidence of PH associated with rheumatic mitral valve disease (PH-MVD) increases with increasing duration of disease and is associated with poor prognosis.[6] Nevertheless all patients with rheumatic mitral valve disease do not develop PH suggesting possible genetic susceptibility for its development. Although the role of genetics in group I PH has been extensively investigated, there is scant data on the genetic influences in group II PH. The mutations in various genes including bone morphogenetic protein receptor type 2 (BMPR2), activin A receptor type II like kinase-1 (ALK1), endoglin, sterile alpha motif domain containing 9 (SMAD9), caveolin-1 (CAV1) and potassium channel subfamily K member 3 (KCNK3) have been reported to be associated with PAH. However, there are no data regarding their role in PH-MVD.[7] We have previously shown that endothelin-1 (ET-1) gene and endothelin receptor A (ET_A) gene polymorphisms, are significantly more prevalent in patients with PH associated with rheumatic mitral valve disease.[8]

There is increased expression of endothelin-1 in the pulmonary vascular cells in patients with idiopathic pulmonary arterial hypertension suggesting that its mitogenic, inflammatory, angiogenic and vasoconstrictor actions play a role in disease expression.[9] ET-1 levels have been variably reported in patients with pulmonary hypertension with some studies describing elevated levels correlating with disease severity while others showing no change in blood levels.[10,11] Allen et al studied 13 children with pulmonary hypertension and reported that immunoreactive ET-1 levels were elevated and strongly correlated with pulmonary vasoreactivity during acute hypoxia.[10] Cernacek et al reported that endothelin levels were normal in stable heart failure patients whereas they were raised in patients with cardiogenic shock as well as in pulmonary hypertension.[11] However, Bacakoglu et al reported that plasma levels of ET-1 did not significantly differ between chronic obstructive pulmonary disease patients with or without pulmonary hypertension.[12]

Endothelin-1 levels have been shown to have a direct association with the risk of coronary atherosclerosis.[13] Also, the T allele of Lys198Asn polymorphism of ET-1 gene has been reported to be a risk factor for coronary atherosclerosis.[13] In a meta-analysis including 1291 cases and 2513 controls, Lys198Asn polymorphism of ET-1 gene was associated with an increased risk of large vessel ischemic stroke.[14] These data show that ET-1 is involved in multiple vascular diseases. Further, administration of endothelin receptor blockers reduces pulmonary vascular resistance and improves symptoms and prognosis in patients with idiopathic PAH.[15] Although rheumatic heart disease (RHD) is 1.5–2 times more common in females compared with males,[16] it is unknown whether female patients with RHD are genetically more predisposed to develop pulmonary hypertension.

We aimed to investigate whether a difference in the distribution of genotypes existed in endothelin-1 and endothelin receptor A genes between female and male patients of pulmonary hypertension associated with rheumatic mitral valve disease.

2. Methods

We enrolled 246 subjects, 123 consecutive PH-MVD subjects (Group A) and 123 age and sex matched healthy controls (Group B), aged ≥18 years from the outpatient Department of Cardiology in this cross-sectional study. The study was done from December 2016 to March 2020 at the first author's tertiary care academic institution. Group A subjects had isolated chronic rheumatic mitral valve stenosis with associated PH [right ventricular systolic pressure (RVSP) ≥36 mm Hg]. Only subjects on regular rheumatic prophylaxis with no acute rheumatic fever history in the past one-year and having no other significant valvular lesion were included. The study was approved by Institutional Ethics Committee and each subject provided written informed consent.

Echocardiography Examination- The study subjects underwent detailed transthoracic two-dimensional echocardiography, M-mode and Doppler examination for the evaluation of valvular heart disease along with left and right ventricular function. The study was performed in a resting, non-sedated state in the left lateral decubitus position. M-mode echocardiography through the mitral valve from the parasternal long-axis view was done to determine the left atrial diameter, interventricular septum and posterior wall thickness, left ventricular (LV) end-diastolic and end-systolic dimensions. The apical four-chamber and two-chamber views were used to determine LV ejection fraction. Mitral valve area was calculated by planimetry and pressure-half time method. An average of 5 readings was taken. Right ventricular dimensions and function were determined by measuring RV basal and mid-cavity diameters and tricuspid annular plane systolic excursion (TAPSE). RVSP was calculated from peak tricuspid regurgitation (TR) jet obtained under color flow imaging guidance using formula, $RVSP = 4(V_{TR})^2 + RAP$, where V_{TR} is peak velocity of TR jet and RAP is estimated right atrial pressure. The pulmonary artery end-diastolic pressure (PADP) was calculated from pulmonary regurgitation jet continuous wave Doppler trace.[1,8]

Blood Investigations and Genotyping- The hematology and biochemistry tests, anti-streptolysin O (ASO) titers, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), endothelin-1 levels and DNA extraction were done from peripheral blood. Serum and anti-coagulated blood samples were labeled and stored at −80 °C (Eppendorf, USA). The DNA was extracted from peripheral blood leukocytes using DNA extraction kit (Gene Aid, India) following the manufacturer's protocol. Two polymorphisms, Lys198Asn (rs5370) on ET-1 gene and His323His (rs5333) on ET_A gene, were studied and analyzed by polymerase chain reaction restriction fragment length polymorphism (PCR RFLP).[8] The primers and restriction enzymes used for ET-1 and ET_A gene polymorphisms were as previously reported.[8] The *Lys198Asn* PCR product was a 116 base pair (bp) fragment, while the *His323His* PCR product was a 154 bp fragment. The restriction digestion of the PCR amplicons was done at 37 °C for 15 min and heat inactivation at 65 °C for 5 min with fast digest restriction enzymes Nhe*I* and BspT*I* (Thermo Scientific, USA) for study of Lys198Asn and His323His polymorphisms respectively. The Lys and Asn alleles of Lys198Asn polymorphism correspond to 116bp and 97bp+19bp sized fragments (Fig. 1A), and in His323His (C/T) polymorphism, the alleles C and T corresponds to 154bp and 87bp+67bp sized fragments respectively (Fig. 1B). The digested products were visualized on 5% agarose gel electrophoresis after staining with ethidium bromide under gel documentation system (Bio Rad, USA). Serum endothelin-1 levels were measured by ELISA using human endothelin-1 ELISA kit (Bioassay Technology Laboratory, China).

3. Statistical analysis

The demographics, biochemical and echocardiographic parameters are expressed as mean ± standard deviation. Range and median are provided for demographics and biochemistry tests. The goodness of fit test was applied to adequately describe the data. The normality of distribution was determined using Shapiro–Wilk test. The p value was calculated using Kruskal–Wallis test. Chi-square, χ^2 test was performed to compare the genotypes and allele frequencies of Lys198Asn and His323His polymorphisms among the

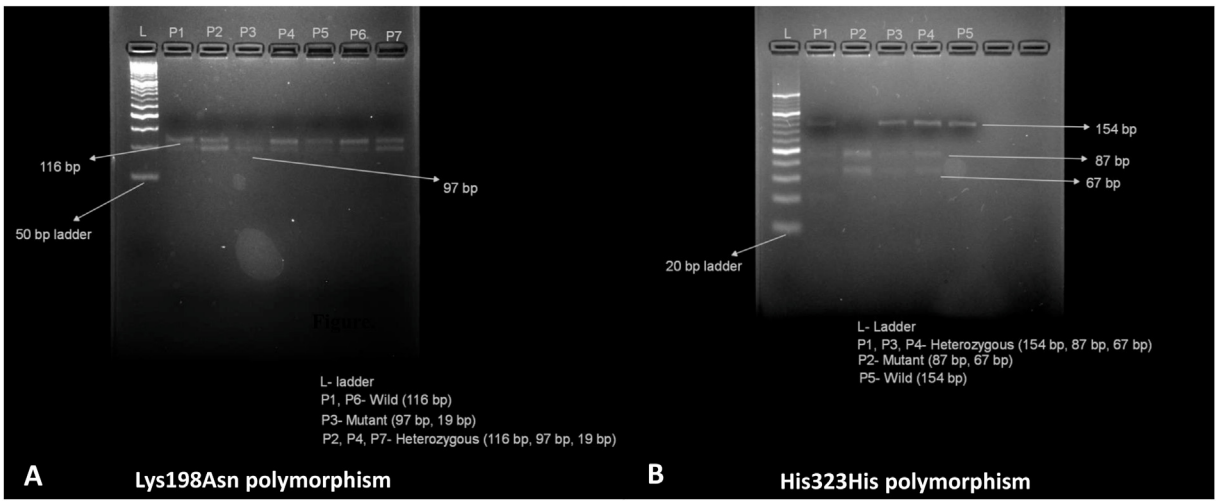

Fig. 1. (A) Analysis of the Lys198Asn polymorphism in the endothelin-1 (ET-1) gene. Restriction fragments are shown after digestion with Nhe*I* **(B)** Analysis of the His323His polymorphism in the endothelin receptor A (ETA) gene. Restriction fragments are shown after digestion with BspT*I*. bp: base pairs; P: patients.

groups. The genotype distribution along with demographics, echocardiography and biochemical parameters were compared among the groups using the multiple logistic regression analysis to ascertain the independent effect of each variable or risk factor on the likelihood of the pulmonary hypertension. Odd's risk estimates were calculated for both the polymorphisms. A p value of <0.05 was considered statistically significant. SPSS version 26 software was used for statistical analyses.

4. Results

The baseline characteristics of both the groups are given in Table 1. The female subjects in the Group A were slightly older with a median age of 35 years while the median age of males was 32 years. Females had higher body mass index than males with a median body mass index of 20.45 kg/m^2 vs 19.5 kg/m^2. Blood anti-streptolysin O titers were <200 IU and C-reactive protein was non-reactive in both Group A and B subjects suggesting absence of acute rheumatic activity. Atrial fibrillation was present in 42% males and 31% females in Group A, while all patients in Group B had normal sinus rhythm. Both males and females in Group A had severe mitral stenosis with severe PH and had New York Heart Association Class II-III dyspnea. Male patients had lower mitral valve area on planimetry (p = 0.037) but similarly higher RVSP (p = 0.112) compared with females. The male patients in Group A had larger RV basal and mid-cavity diameters (p < 0.0001 for both) (Fig. 2). Echocardiography showed enlarged right atrium (RA) with markedly decreased RA fractional area change signifying RA dysfunction in both genders in Group A. The tricuspid annular plane systolic excursion (TAPSE) was significantly decreased in both males and

Table 1
Baseline characteristics according to gender in both the study groups.

Baseline parameters	Group A n = 43 (Males)	Median	Range	Group A n = 80 (Females)	Median	Range	Group B n = 47 (Males)	Median	Range	Group B n = 76 (Females)	Median	Range	p value
Age (years)	32.8 ± 10.9	32	18–60	38.1 ± 9.2	35	18–57	34.1 ± 9.2	33	18–60	36.7 ± 9.5	35.5	20–54	0.298
Weight (kg)	49.1 ± 2.8	49.6	43–60	49.1 ± 2.3	49	38–57	60.5 ± 9.9	60.2	40–85	57.5 ± 8.7	58.35	41.2 –76.1	<0.0001*
Height (cm)	158.9 ± 7.2	158	149 –184	156.5 ± 5.9	156	141 –168	160.5 ± 5.6	161	148 –174	158.6 ± 5.6	158	143 –172	<0.0001*
BMI (kg/m^2)	19.5 ± 1.4	19.5	17.1 –22.5	20.1 ± 1.7	20.45	16.8 –24.2	23.4 ± 3.1	23.3	17.3 –29.8	22.8 ± 3.3	23.35	16.5 –29.7	<0.0001*
Heart rate (beats/ min)	79.1 ± 5.5	80	50–88	79.0 ± 3.96	80	60–88	79.1 ± 4.2	80	66–95	80.5 ± 7.2	80	70–115	0.269
ECG (sinus rhythm: AF) n (%)	25 (58%): 18(42%)	–	–	55 (69%):25 (31%)	–	–	47 (100%): 0 (0%)	–	–	76 (100%): 0 (0%)	–	–	–
Systolic BP (mm Hg)	127 ± 8.2	128	110 –140	123 ± 9.4	128	100 –138	126 ± 6.5	128	110 –140	124 ± 7.6	128	110 –138	0.884
Diastolic BP (mm Hg)	78.4 ± 3.7	80	70–88	77.5 ± 5.3	80	60–88	78.8 ± 2.6	80	70–80	78.1 ± 4.1	80	60–86	0.45
Hemoglobin (gm/dl)	13.5 ± 1.8	13.3	10–18	12.2 ± 1.8	11.99	9–16.6	14.6 ± 1.2	14.7	10.5 –17.2	12.1 ± 1.4	12	9–14.8	<0.0001*
ESR (mm/hr)	16.7 ± 12.2	15	2–60	21.4 ± 17.8	16.5	2–85	9.8 ± 7.5	8	2–40	16.6 ± 15.0	15	5–80	<0.0001*
Blood urea (mg/dl)	22.5 ± 3.3	23	14–28	23.0 ± 4.3	22	11–29	23.2 ± 5.3	23	13–37	22.2 ± 5.7	21	12–39	0.68
Serum creatinine (mg/dl)	0.79 ± 0.09	0.8	0.6–0.9	0.77 ± 0.09	0.8	0.6–0.9	0.79 ± 0.09	0.8	0.6–0.9	0.72 ± 0.08	0.7	0.5–0.9	<0.0001*
Serum endothelin-1 levels (ng/L)	87.6 ± 181.1	30.44	5.18 –911.67	110.9 ± 171.7	68.57	6.51 –934.83	134.9 ± 143.4	95.71	0.34 –684.28	117.5 ± 92.9	96.39	9.99 –816.15	0.482

*significant. p-value has been calculated using Kruskal–Wallis test.
AF: atrial fibrillation; BMI: body mass index; BP: blood pressure; ESR: erythrocyte sedimentation rate.

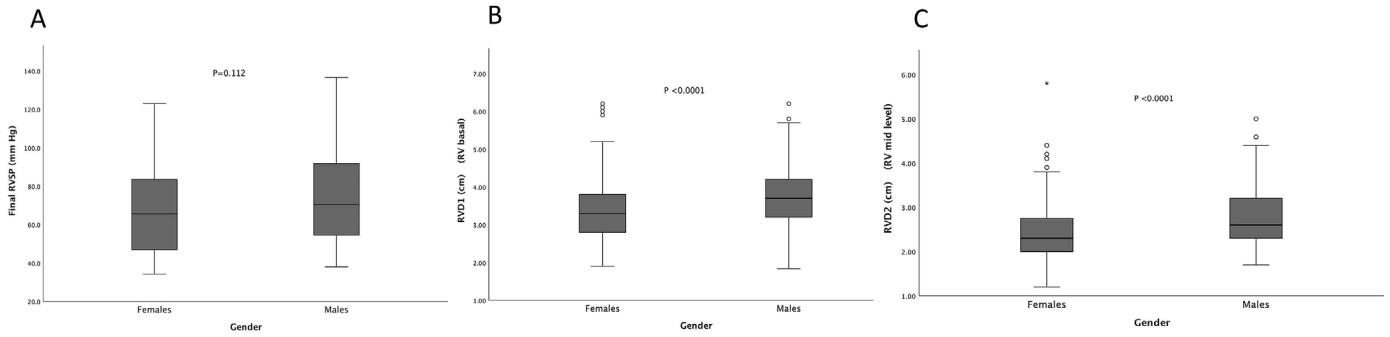

Fig. 2. Regression variable plots showing gender differences in **(A)** right ventricular systolic pressure (RVSP) **(B)** right ventricular basal diameter (RVD1), and **(C)** right ventricular mid-cavity diameter (RVD2) in group A subjects with pulmonary hypertension associated with rheumatic mitral valve disease. The box plots show that despite having similarly high RV systolic pressures, males have significantly more RV dilatation.

females in Group A compared to Group B ($p < 0.0001$) suggesting borderline RV systolic dysfunction (Table 2). The subjects in Group B were asymptomatic healthy controls and had normal echocardiography examination.

The mutant homozygous Asn/Asn genotype in Lys198Asn and T/T genotype in His323His polymorphism was more prevalent in Group A (reported previously).[8] When Group A subjects were segregated with respect to gender, the genotype frequency was different in male and female subjects. The mutant and heterozygous genotypes and allele frequencies were more commonly present in females compared to males in Lys198Asn ($p = 0.02$) and His323His polymorphisms ($p = 0.01$) (Table 3). The mutant Asn allele frequency was more common in Group A than Group B (0.5 vs 0.36). Also, the mutant T allele was more common in Group A than Group B. The presence of mutant Asn/Asn and either mutant Asn/Asn or heterozygous Lys/Asn genotypes of Lys198Asn polymorphism when compared to wild Lys/Lys genotype in females showed a significant association with higher risk (odds ratio [OR] 4.5; 95% confidence interval [CI] 1.44–14.04 and OR 2.39; 95% CI 1.15–4.96, respectively). The presence of heterozygous C/T and either mutant T/T or heterozygous C/T genotypes of His323His polymorphism when compared to wild C/C genotype in females showed a significant association with higher risk (OR 1.96; 95% CI 1.0–3.85 and OR 2.26; 95% CI 1.17–4.38, respectively). No significant difference was seen in genotypic frequencies in males (Table 4).

The logistic regression model was statistically significant, $\chi^2 = 21.676$, $p < 0.05$. The model explained 17.3% (Nagelkerke R^2) of the variance. The Hosmer–Lemeshow tested the null hypothesis that predictions made by the model fit perfectly with the observed group. Logistic regression analysis included demography, echocardiography, biochemical parameters and genotype frequencies of ET-1 and ET_A gene polymorphisms. The analysis revealed that in Group A, enlarged LA ($p < 0.0001$), increased posterior wall thickness in diastole ($p = 0.004$), enlarged main pulmonary artery ($p < 0.0001$), dilated inferior vena cava ($p = 0.02$), mutant genotype Asn/Asn ($p = 0.007$) and heterozygous genotype Lys/Asn of Lys198Asn polymorphism ($p = 0.018$) were independent predictors

Table 2
Baseline echocardiography parameters according to gender in both the study groups.

Echocardiography parameters	Group A $n = 43$ (Males)	Group A $n = 80$ (Females)	Group B $n = 47$ (Males)	Group B $n = 76$ (Females)	p value
Left atrial diameter (cm)	5.2 ± 0.96	4.9 ± 0.8	3.15 ± 0.52	2.96 ± 0.44	<0.0001*
LV internal dimension in diastole (cm) (LVIDd)	4.56 ± 1.00	4.31 ± 0.67	4. 6 ± 0.35	4.21 ± 0.45	0.004*
LV internal dimension in systole (cm) (LVIDs)	3.13 ± 0.78	2.93 ± 0.59	3.06 ± 0.4	2.78 ± 0.45	0.021*
Posterior wall thickness in diastole (cm)	0.84 ± 0.13	0.89 ± 0.17	0.88 ± 0.13	0.84 ± 0.12	0.157
IVS thickness in diastole (cm)	0.92 ± 0.7	0.87 ± 0.14	0.86 ± 0.09	0.86 ± 0.12	0.133
Right ventricular wall thickness in diastole (cm)	0.51 ± 0.11	0.62 ± 0.74	0.42 ± 0.40	0.36 ± 0.04	<0.0001*
Main pulmonary artery diameter (cm)	2.79 ± 0.46	2.65 ± 0.58	2.14 ± 0.24	1.97 ± 0.16	<0.0001*
LV ejection fraction (%)	54.97 ± 6.32	56.9 ± 5.34	59.00 ± 4.93	58.1 ± 4.6	0.019*
Right atrial major dimension (cm)	5.81 ± 1.3	5.66 ± 1.3	4.14 ± 0.44	4.09 ± 0.51	<0.0001*
Right atrial end-systolic area (cm^2)	15.14 ± 9.15	13.12 ± 9.35	6.5 ± 1.64	5.63 ± 1.4	<0.0001*
Right atrial end-diastolic area (cm^2)	19.38 ± 8.48	17.8 ± 10.02	12.71 ± 2.3	11.31 ± 2.03	<0.0001*
Right atrial fractional area change (FAC) (%)	29.80 ± 16.83	29.7 ± 13.4	49.32 ± 7.74	50.06 ± 9.6	<0.0001*
IVC diameter (inspiration) (cm)	0.93 ± 0.42	0.82 ± 0.4	0.56 ± 0.14	0.54 ± 0.13	<0.0001*
IVC diameter (expiration) (cm)	1.64 ± 0.47	1.64 ± 0.45	1.44 ± 0.26	1.4 ± 0.3	<0.0001*
RV basal diameter (cm)	4.22 ± 0.90	3.80 ± 0.93	3.36 ± 0.35	3.12 ± 0.45	<0.0001*
RV mid cavity diameter (cm)	3.24 ± 0.75	2.6 ± 0.71	2.4 ± 0.36	2.07 ± 0.32	<0.0001*
TAPSE (cm)	1.73 ± 0.31	1.8 ± 0.3	2.02 ± 0.13	1.98 ± 0.19	<0.0001*
RVOT VTI (cm) (average)	14.18 ± 4.47	17.4 ± 3.63	20.39 ± 2.96	19.67 ± 2.55	<0.0001*
Mitral valve area by planimetry (cm^2)	0.76 ± 0.17	0.88 ± 0.16	–		0.037*
Mitral valve area by pressure half-time (cm^2)	0.75 ± 0.15	0.85 ± 0.18	–		0.047
Peak diastolic transmitral gradient (mm Hg)	20.23 ± 7.03	18.13 ± 5.42	–		0.139
Mean diastolic transmitral gradient (mm Hg)	12.25 ± 4.93	10.74 ± 3.96	–		0.294
Right ventricular systolic pressure (mm Hg)	75.00 ± 24.97	65.5 ± 22.1	–		0.112
Pulmonary artery end-diastolic pressure (mm Hg)	26.25 ± 9.93	25.64 ± 10.64	–		0.740
Pulmonary artery mean pressure (mm Hg)	32.96 ± 15.13	38.99 ± 13.75	–		0.911

*significant.
IVC: inferior vena cava; IVS: interventricular septum; LV: left ventricular; *n*: number of study subjects; PH-MVD: pulmonary hypertension associated with rheumatic mitral valve disease; RV: right ventricular; RVOT VTI: right ventricular outflow tract velocity time integral; TAPSE: tricuspid annular plane systolic excursion.

Table 3
Frequency of ET-1 and ET_A gene polymorphism in Group A and Group B subjects based on their gender specific distribution.

Subjects	Lys/Lys	Asn/Asn	Lys/Asn	Lys allele	Asn allele	Chi square, χ^2	*p* value
Group A (Males) ($n = 43$), *n* (%)	9 (20.9%)	9 (20.9%)	25 (58.1%)	0.5	0.5	2.82	0.24
Group B (Males) ($n = 47$), *n* (%)	12 (25.5%)	4 (8.5%)	31 (65.9%)	0.59	0.41		
Group A (Females) ($n = 80$), *n* (%)	15 (18.8%)	15 (18.8%)	50 (62.5%)	0.5	0.5	7.71	0.02*
Group B (Females) ($n = 76$), *n* (%)	27 (35.5%)	6 (7.9%)	43 (56.6%)	0.64	0.36		
Subjects	**C/C**	**T/T**	**C/T**	**C allele**	**T allele**	**Chi square, χ^2**	***p* value**
Group A (Males) ($n = 43$), *n* (%)	26 (60.5%)	1 (2.3%)	16 (37.2%)	0.79	0.21	1.09	0.57
Group B (Males) ($n = 47$), *n* (%)	28 (59.6%)	0 (0%)	18 (38.3%)	0.80	0.20		
Group A (Females) ($n = 80$), *n* (%)	42 (52.5%)	5 (6.25%)	33 (41.3%)	0.73	0.27	8.88	0.01*
Group B (Females) ($n = 76$), *n* (%)	55 (72.4%)	0 (0%)	22 (28.9%)	0.86	0.14		

* significant. *p*-value has been calculated using Chi square test.
Lys/Lys: wild-type, Asn/Asn: homozygous variant, Lys/Asn: heterozygous variant of Lys198Asn polymorphism; C/C: wild-type, T/T: homozygous variant, C/T: heterozygous variant of His323His polymorphism; *n*: number of study subjects; χ^2 = Chi square; ET-1: endothelin-1; ET_A: endothelin receptor type A.

Table 4
Odds risk estimates of ET-1 and ET_A gene polymorphism genotypes in Group A compared to Group B based on their gender specific distribution.

Gene polymorphism/SNP ID	Genotypes compared	Group A $n = 123$, *n* (%)	Group B $n = 123$, *n* (%)	Odds ratio (OR)	95% confidence interval (CI)	*p* value
Lys198Asn (rs5370)	**Genotypes compared (Males)**	**Group A (Males), n = 43**	**Group B (Males), n = 47**	**OR**	**95% CI**	***p* value**
	Asn/Asn *vs* Lys/Lys	9 (20.9%)	4 (8.5%)	3	0.69–12.92	0.13
	Lys/Asn *vs* Lys/Lys	25 (58.1%)	31 (65.9%)	1.07	0.39–2.95	0.89
	Asn/Asn + Lys/Asn *vs* Lys/Lys	34 (79%)	35 (74.5%)	1.29	0.48–3.46	0.60
	Genotypes compared (Females)	**Group A (Females), n = 80**	**Group B (Females), n = 76**	**OR**	**95% CI**	***p* value**
	Asn/Asn *vs* Lys/Lys	15 (18.8%)	6 (7.9%)	4.5	1.44–14.04	0.007*
	Lys/Asn *vs* Lys/Lys	50 (62.5%)	43 (56.6%)	2.09	0.98–4.44	0.05
	Asn/Asn + Lys/Asn *vs* Lys/Lys	65 (81.3%)	49 (64.5%)	2.39	1.15–4.96	0.02*
Polymorphism	**Genotypes compared (Males)**	**Group A (Males), n = 43**	**Group B (Males), n = 47**	**OR**	**95% CI**	***p* value**
His323His (C/T)[a] (rs5333)	C/T *vs* C/C	16 (37.2%)	18 (38.3%)	0.95	0.40–2.26	0.92
	T/T + C/T *vs* C/C	17 (39.5%)	18 (38.3%)	1.02	0.43–2.38	1
	Genotypes compared (Females)	**Group A (Females), n = 80**	**Group B (Females), n = 76**	**OR**	**95% CI**	***p* value**
	C/T *vs* C/C	33 (41.3%)	22 (28.9%)	1.96	1.00–3.85	0.047*
	T/T + C/T *vs* C/C	38 (47.5%)	22 (28.9%)	2.26	1.17–4.38	0.01*

*significant.
[a] Odds risk ratio of T/T genotype could not be determined as control group did not have representation of mutant homozygous T/T genotype.

of development of PH in females. In Group A males, enlarged LA ($p = 0.02$), enlarged main pulmonary artery ($p < 0.0001$), dilated inferior vena cava ($p = 0.003$) predicted the development of PH.

5. Discussion

In this study recessive genotype Asn/Asn (Lys198Asn) of ET-1 gene and T/T genotype (His323His) of ET_A gene polymorphisms were significantly more prevalent in females than males in PH-MVD patients (Group A) as compared to healthy controls (Group B). We studied two polymorphisms: (1) Lys198Asn polymorphism which is G to T transversion at location 5665 affecting 61st nucleotide of exon 5 of ET-1 gene which results in different biological ET-1 isoforms. The genotypes of this polymorphism are, wild-type: Lys/Lys (GG), heterozygous: Lys/Asn (GT), and variant: Asn/Asn (TT).[17] (2) His323His (C/T) polymorphism located at 6th exon of ET_A gene on chromosome 4, substitutes thymine (T) for cytosine (C). The genotypes are: wild-type: C/C, heterozygous: T/C, and variant: T/T.[18]

Although the group I PH has been extensively investigated for genetic causes, the data for genetic influences in group II PH is sparse. It is important to study the genetic predisposition so as to better understand the pathophysiology and explore newer therapeutic targets. Further patients of PAH with genetic mutation have poorer prognosis compared to those without mutation. The pathophysiological role of the studied polymorphisms in idiopathic PAH has been reported.[19,20] We also described previously that ET-1 and ET_A gene polymorphisms are more common in PH associated with rheumatic mitral valve disease.[8]

The female subjects are more prone to develop pulmonary arterial hypertension.[2] A greater percentage of PAH patients in several registries are females thus suggesting that female gender may be a risk factor for PAH.[2] Also, connective tissue disorder associated pulmonary hypertension has a 9:1 female to male ratio.[3] Although various mechanisms have been proposed to explain the differences in the incidence, pathophysiology and prognosis between men and women with PAH, including smaller airway dimensions in females, sex hormones, immune-mediated mechanisms, genetic factors, comorbidities and social factors, genetic predisposition in combination with environmental factors probably plays a major role in disease expression.[2,21] The Screening of pulmonary arterial hypertension in asymptomatic bone

morphogenetic protein receptor 2 (BMPR2) mutation carriers (DELPHI-2) study showed that PAH incidence in BMPR2 mutation carriers was more than three times higher in females than males (3.5% per year in women and 0.99% per year in men) suggesting that females have a predilection to develop the disease.[22,23] However the carriers of BMPR2 mutations do not inevitably develop PAH, though the risk of development of PAH is markedly increased in asymptomatic carriers of BMPR2 mutations compared to non-carriers, with PAH developing in about 42% women and 14% men who inherit BMPR2 mutation.[24] This incomplete penetrance, variable expressivity, and female predominance of PAH suggests that genetic and other factors modify disease expression.[7]

The female human pulmonary artery smooth muscle cells (hPASMCs) are reported to have decreased BMPR2 signaling compared with male hPASMCs. The estrogen-driven suppression of BMPR2 signaling likely contributes to a pro-proliferative phenotype in female hPASMCs predisposing women to PAH.[25] Further, polymorphisms in the aromatase gene associated with increased estradiol production have also been associated with increased risk of porto-pulmonary hypertension in patients with liver disease.[26]

The concurrent influence of genetic predilection on underlying disease or drug exposure in the development of PH is exemplified by many examples. The BMPR2 mutation carriers developed PH after a significantly shorter exposure time to fenfluramine compared to non-carriers with PH associated with fenfluramine.[27] Further the genetic defect itself may influence the severity. The PAH associated with mutations in the activin A receptor type II like kinase-1 (ALK1), a receptor in the transforming growth factor-β receptor family, is more severe compared to BMPR2 mutations.[28] This suggests that both genetic predisposition and underlying condition influences the development of PH and its prognosis.

Right ventricular function is a major determinant of functional status and survival in pulmonary hypertension. Normal female subjects have better RV function than males which persists even after multivariate adjustment for LV function and body size. In PAH also, females have better RV function and improved survival compared to males.[29] Normally estrogen has direct, receptor-mediated RV cardioprotective effects. However, it has been proposed that vasoprotective estrogen signaling becomes maladaptive in the setting of genetic predisposition or deleterious environmental factors.[30] There are no previous studies assessing gender differences in RV function in PH associated with RHD. Our study shows that despite having severe PH and similar RV systolic pressure and pulmonary artery end-diastolic pressures, RV basal and mid cavity diameters were significantly higher in males, with lower right ventricular outflow tract velocity time integral. Thus, males with PH-MVD exhibited more RV cavity dilatation and lower RV systolic function compared with females.

In our study the ET-1 and ET_A gene polymorphisms were more common in females than males in PH-MVD suggesting possible role in the development of PH in patients with RHD. The functional significance of these polymorphisms and mechanisms by which they may affect the pulmonary vasculature need further study.

6. Limitations

This is an initial study exploring whether there are gender differences in ET-1 and ET_A gene polymorphisms thereby increasing the susceptibility to develop PH in rheumatic MVD patients. Larger studies are required to corroborate our findings. Moreover in vitro studies investigating the functional role of these polymorphisms in the pathogenesis of PH are warranted. The disease-modifying effects or gene–gene interactions of the studied polymorphisms need to be elucidated. These require further study.

7. Conclusions

This preliminary study for the first time shows that ET-1 and ET_A gene polymorphisms were more common in females than males in subjects with PH-MVD. These polymorphisms may act as disease modifiers, and may influence the development of PH by interacting with other genes and environmental factors. However, this requires confirmation in further studies.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: VIMAL MEHTA reports financial support was provided by SERB, Department of Science & Technology, Govt of India.

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