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Comparison Of Early Left Ventricular Hypertrophy Regression Between Metallic Versus Tissue Valve In Aortic Valve Replacement In Patient With Aortic Valve Disease

Tania Nusrat Shanta[1], Cm Mosabber Rahman[2], Mirza Md. Nazmus Saquib[3], Muhammad Abul Kalam[4], Shakila Yeasmin[5], Nadia Zaman[6], ShohanaShobnam Mouly[7], Mohammad Alauddin[8], Rezwanul Haque[9]

[1]specialist Cardiac Surgeon, Department Of Cardiac Surgery, United Hospital Limited, Dhaka, Bangladesh
[2]junior Consultant, Department Of Cardiac Surgery, Dhaka Medical College Hospital, Dhaka, Bangladesh.
[3]director, Nexus Cardiac Hospital & Research Ltd., Mymensingh, Bangladesh.
[4]surveillance Medical Officer (Smo), National Malaria Elimination Program (Nmep), Communicable Disease Control (Cdc) Operational Plan, Directorate General Of Health Services (Dghs), Mohakhali, Dhaka, Bangladesh
[5]monitoring & Evaluation Officer. Ntp (National Tuberculosis Control Program), Dghs.
[6]medical Officer, Covid-19 Emergency Response And Pandemic Preparedness (Erpp) Project, Directorate General Of Health Services (Dghs), Mohakhali, Dhaka, Bangladesh
[7]medical Officer, Covid-19 Emergency Response And Pandemic Preparedness (Erpp) Project, Directorate General Of Health Services (Dghs), Mohakhali, Dhaka, Bangladesh
[8]consultant, Department Of Cardiac Surgery, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[9]professor, Department Of Cardiac Surgery Bangabandhu Sheik Mujib Medical University, Dhaka, Bangladesh

Abstract
Introduction: *The prevalence ofAortic valve disease is rising in Bangladesh, making aortic valve replacement surgery as one of the most frequently performed surgeries.*
Aim of the study:*The aim of this study was to evaluate left ventricular mass reduction and left ventricular functional status after aortic valve replacement with different valve substitutes; metallic versus tissue valves.*
Methods:*This comparative cross-sectional study was conducted in Department of Cardiac Surgery, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka-1000, Bangladesh from August, 2020 to July, 2022. Total 60 patient with aortic valve disease were included in this study. These patients were assigned into two groups. Each group consisted equal numbers of patients. Group-A patients received metallic valve whereas Group-B patients received tissue valve.*
Result:*The predominant age groups in Group-A and Group-B were 51-60 years and >70 years, respectively, with no significant difference in mean ages (55.1 ± 10.96 years vs. 61.7 ± 16.91 years). Gender distribution was also not significantly. Echocardiographic variables showed no significant pre-operative differences. Post-operative changes at 1 and 3 months revealed significant alterations in LVESD, LVEF, IVST, PWT and LV mass index in Group-A, and inLVESD, IVST, PWT, and LV mass index in Group-B. Regression of LV mass index post-operatively was more pronounced in Group-A (24.48% at 1 month, 36.16% at 3 months) compared to Group-B (14.74% at 1 month, 22.86% at 3 months).*
Conclusion:*This study observed superior left ventricular mass regression after aortic valve replacement with metallic valve in comparison to tissue valve.*
Key words:*early left ventricular hypertrophy regression, metallic valve, tissue valve*

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I. Introduction

Prevalence of moderate to severe valve disease is 8.5% in 65-74 years and 13% in those over 75 years as seen in the study.[1] The number of rheumatic heart disease in Southeast Asia was 28 per 1000 as shown in a more recent meta-analysis in echocardiographically diagnosed rheumatic heart disease.[2] Similarly, prevalence of aortic stenosis in elderly population is 20.9%.[3] In Bangladesh, 34% of all cardiac admissions in the hospitals are with rheumatic heart disease.[4]The only definitive treatment for most patients with severe valvular heart disease is prosthetic heart valve replacements and about 4 million prosthetic heart valve replacements have been done over the past 50 years.[5] The total number of replacements is projected to be 8,50,000 per year by 2050.[6] The etiology of aortic stenosis (AS) is degenerative-calcification in the majority of patients however a number of other potential pathophysiologies have been invoked including atherosclerosis, calcific, genetic and pathological investigations have shown that with age, collagen of the valve leaflets is destroyed, and calcium is deposited on the leaflets.[7] In aortic regurgitation, a large volume of blood is regurgitated into the left ventricle in each diastole. The left ventricular output may be more than doubled. The increased stoke volume necessary to achieve this dilatation of the left ventricle. The amount of blood that regurgitates is largely determined by the severity of aortic valve disease but also influenced by the compliance of the left ventricle and the systemic vascular resistance.[8]Left ventricular (LV) hypertrophy is a form of adaptation to a chronic cardiac overload. In the setting of aortic stenosis (AS), LV hypertrophy develops to limit the increase of LV systolic wall stress. Aortic valve replacement (AVR), is expected to eliminate the LV burden, inducing regression of LV hypertrophy.[9] Both aortic stenosis (AS) and aortic regurgitation (AR) are common aortic valve disease and frequently have left ventricular dysfunction.[10] Severe aortic stenosis is often associated with concentric hypertrophy of left ventricle which is caused by a high afterload. Similarly, severe aortic regurgitation mainly induces the left ventricular volume progression. More than one fourth with aortic regurgitation showed symptoms.[11]The usual symptoms are dyspnea, angina or orthopnea. Both in severe aortic stenosis and aortic regurgitation patients will deteriorate into irreversible myocardial dysfunction such as left ventricular heart failure which may increases the risk of sudden death. Aortic valve replacement (AVR) is an effective therapy which may reduce the potential risk of sudden death and improve left ventricular function.[12]As a result of pressure gradient across the aortic valve, left ventricular pressure must rise in order to maintain a normal perfusion pressure in the ascending aorta. This increases the left ventricular pressure and left ventricular wall stress during systole. In term, increased wall stress is thought to be the stimulant for left ventricular hypertrophy which ultimately normalizes the wall stress by increasing wall thickness. Aortic valve replacement is the standard therapy for patients with significant aortic valve disease and there has been persistent improvement in hemodynamic status following AVR.[13] After aortic valve replacement (AVR) for aortic regurgitation, left ventricular volume and mass decrease with improvement in left ventricular filling, although this can be delayed.[14]The purpose of this study is to observe the effect of aortic valve replacement on left ventricular mass regression in patients with aortic valve disease, by using metallic and tissue valve.

II. Objectives

General Objective:

- To compare pre- and post-operative LVH regression both in metallic and tissue valve based on echocardiographic findings of the patient after aortic valve replacement (AVR).

Specific objectives

- Echocardiographic assessment of the post-operative LV mass regression after AVR with metallic valve.
- Echocardiographic assessment of the post-operative LV mass regression after AVR with tissue valve.
- To study the effect of AVR on global post-operative left ventricular function as measured by left ventricular ejection fraction.
- To compare pre- and post-operative left ventricular tissue mass index (gm/m^2)

III. Methodology & Materials

This comparative cross-sectional study was conducted inDepartment of Cardiac Surgery, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka-1000, Bangladesh, during the period from August, 2020 to July, 2022. Total 60 patient with isolated aortic stenosis, Aortic regurgitation and mixed lesion, who underwent aortic valve replacement with prosthetic valve were included in this study. The patients were divided into two groups - group A: thirty (30) patients who had aortic valve replacement with metallic valve and group B: thirty (30) patients who had Aortic valve replacement with tissue valve. Ethical approval was taken from Institutional Review Board (IRB). Informed written consent was taken from each individual before starting the data collection. A standardized semi-structured data collection tool was used to collect necessary information of the study subject. After collection of data, all data were checked and cleaned. After cleaning, the data were entered into computer and statistical analysis of the results being obtained by using windows-based computer software devised with Statistical Packages for Social Sciences version 22. P value of less than 0.05 was considered statistically significant.

Inclusion Criteria:

- Patient with isolated aortic stenosis, aortic regurgitation and mixed aortic stenosis and regurgitation who underwent aortic valve replacement.

Exclusion Criteria:

- Patients with double valve replacement.
- Aortic valve replacement with Infective endocarditis.
- Patients of aortic valve replacement with coronary artery bypass graft.
- Aortic valve replacement with root enlargement or stent less bioprosthetic valve.
- Left ventricular ejection fraction < 35 %.
- Aortic valve replacement with chronic renal failure, chronic liver disease, malignancy.

IV. Result

Table-Idemonstrated that majority (12, 40%) of the Group-A subjects falls on 51-60 years age group whereas in Group-B majority (14, 46.7%) of the subjects are in >70 years age group. In Group-A maximum and minimum age was 71 and 32 years respectively and in Group-B maximum and minimum age was 78 and 45 years respectively. However, Mean ± SD age of both group were 55.1 ± 10.96 and 61.7 ± 16.91 years in Group-A and Group-B respectively. The mean age between two groups was statistically not significant (p>0.05). In Group-A male and female subjects were 21 (70%) and 9 (30%) respectively and in Group-B male and female subjects were 23(76.7%) and 7 (23.3%) respectively. However, the sex distribution between two groups was statistically not significant (p>0.05). In Group-A and Group-B, 24(80%) and 20 (66.7%) subjects were in normal BMI range respectively. However, two groups were tested statistically in relation to BMI and it was statistically not significant (p>0.05). Mean BSA of Group-A and Group-B were 1.63 ± 0.14 and 1.64 ± 0.15 m^2 respectively. However, difference in mean BSA between two groups was statistically not significant (p>0.05). Table-II showed, pre-operative mean values of echocardiographic variables (LVEDD, LVESD, LVEF, IVST, PWT and LV mass index) in Group-A and Group-B. Difference of mean values of the variables between two groups were not statistically significant (p>0.05). Table-III showed, pre- and post-operative (1 month and 3 months) mean values of echocardiographic variables (LVEDD, LVESD, LVEF, IVST, PWT and LV mass index) in Group-A subjects. At 1 month, PWT and LV mass index showed significant (p<0.05) changes compared to their pre-operative values. Changes in rest of the variables (LVEDD, LVESD, LVEF and IVST) were not statistically significant (p>0.05). At 3 months, most of the variables (LVESD, LVEF, IVST, PWT and LV mass index) showed significant (p<0.05) changes compared to their pre-operative values. However, changes in LVEDD was not statistically significant (p>0.05). Table-IV showed, pre- and post-operative (1 month and 3 months) mean values of echocardiographic variables (LVEDD, LVESD, LVEF, IVST, PWT and LV mass index) in Group-B subjects. At 1 month, IVST,LVESD and PWT showed significant (p<0.05) changes compared to their pre-operative values. Changes in rest of the variables (LVEDD, LVEF and LV mass index) were not statistically significant (p>0.05). At 3 months, IVST, PWT and LV mass index showed significant (p<0.05) changes compared to their pre-operative values. However, changes in LVEDD, LVESD and LVEF were not statistically significant (p>0.05). Table-V showed, post-operative mean values of echocardiographic variables (LVEDD, LVESD, LVEF, IVST, PWT and LV mass index) at 1 month in Group-A and Group-B. Difference of mean values of PWT was statistically significant (p<0.05). However, changes in mean values of most of the variables (LVEDD, LVESD, LVEF, IVST and LV mass index) between two groups were not statistically significant (p>0.05). Table-VI showed, post-operative mean values of echocardiographic variables (LVEDD, LVESD, LVEF, IVST, PWT and LV mass index) at 3 months in Group-A and Group-B. Difference of mean values of most of the variables (LVESD, LVEF, IVST, PWT and LV mass index) was statistically significant (p<0.05). However, changes in mean values LVEDD between two groups were not statistically significant (p>0.05). Figure-1 and table-VII showed that in Group-A LV mass index was 143.74, 108.54 and 91.76 gm/m^2 in pre-operative and post operative at 1month and 3 months period respectively. In Group-B LV mass index in pre-operative and post operative at 1month and 3 months period were 152.09, 129.68 and 117.33 gm/m^2 respectively. Regression of LV mass index in Group-A was 24.48% at 1 month and 36.16% at 3 months post-operatively compared to their pre-operative values. Regression of LV mass index in Group-B was 14.74% at 1 month and 22.86% at 3 months post-operatively compared to their pre-operative values.

Table-I: Demographic characteristics of the study groups (N=60)

Characteristics	Group A	Group B	P-value
	n_1 (%)	n_2 (%)	
Age (in years)			
<40	2 (6.7)	0 (0)	
41-50	7 (23.3)	2 (6.7)	
51-60	12 (40.0)	4 (13.3)	
61-70	8 (26.7)	10 (33.3)	
>70	1 (3.3)	14 (46.7)	

Mean ± SD	**55.1 ± 10.96**	**61.7 ± 16.91**	0.3140^{ns}
Sex			
Male	21 (70)	23 (76.7)	0.5606^{ns}
Female	9 (30)	7 (23.3)	
BMI (Kg/m^2)			
Underweight (<18.5)	0 (0)	2 (6.7)	0.3176^{ns}
Normal (18.5 to 24.9)	24 (80)	20 (66.7)	
Overweight (25 to 29.9)	4 (13.3)	7 (23.3)	
Obese (≥30)	2 (6.7)	1 (3.3)	
BSA (m^2)			
Mean ± SD	1.63 ± 0.14	1.64 ± 0.15	0.88^{ns}

N= n_{1+} n_2
Data analyzed using unpaired t-test
ns = Not significant

Table-II:Comparison of pre-operative echocardiographic variables between two groups (N=60)

Echocardiographic variables	Group A	Group B	P-value
LVEDD	51.5 ± 5.06	50.6 ± 4.99	0.69^{ns}
LVESD	35.7 ± 5.5	37.1 ± 2.6	0.48^{ns}
LVEF	52.11 ± 12.09	46.51 ± 6.59	0.21^{ns}
IVST	11.8 ± 1.93	12.7 ± 1.16	0.22^{ns}
PWT	11.1 ± 1.1	11.9 ± 1.2	0.14^{ns}
LV Mass Index	143.74 ± 39.78	152.09 ± 30.34	0.6^{ns}

Data expressed as Mean ± SD
Data analyzed using unpaired t-test
ns = Not significant

Table-III: Comparison of pre- and post-operative echocardiographic variables in Group-A (n_1=30)

Echocardiographic variables	Pre-operative	Post operative		P-value	
		1 month	3 months	1 month	3 months
LVEDD	51.5 ± 5.06	48.7 ± 5.03	47.7 ± 4.37	0.23^{ns}	0.09 ^{ns}
LVESD	35.7 ± 5.5	32 ± 4.24	29.6 ± 3.53	0.11^{ns}	0.001^{s}
LVEF	52.11 ± 12.09	57.18 ± 9.55	62.04± 7.89	0.31^{ns}	0.04^{s}
IVST	11.8 ± 1.93	10.3 ± 1.7	9.2 ± 1.03	0.08^{ns}	0.001^{s}
PWT	11.1 ± 1.1	9.6 ± 0.84	8.9 ± 0.99	0.003^{s}	0.0002^{s}
LV Mass Index	143.74 ± 39.78	108.54 ± 31.13	91.76 ± 23.24	0.04^{s}	0.002^{s}

Data expressed as Mean ± SD
Data analyzed using unpaired t-test
ns = Not significant
s = Significant

Table-IV: Comparison of pre- and post-operative echocardiographic variables in Group-B (n_2=30)

Echocardiographic variables	Pre-operative	Post operative		P-value	
		1 month	3 months	1 month	3 months
LVEDD	50.6 ± 4.99	49.5 ± 5.66	48.2 ± 5.71	0.65^{ns}	0.33^{ns}
LVESD	37.1 ± 2.6	35 ± 2.49	33.7 ± 2.5	0.08^{ns}	0.008^{s}
LVEF	46.51 ± 6.59	49.91± 8.61	50.99 ± 8.77	0.33^{ns}	0.21^{ns}
IVST	12.7 ± 1.16	11.5 ± 1.08	11 ± 0.82	0.02^{s}	0.001^{s}
PWT	11.9 ± 1.2	10.9± 0.88	10.5 ± 0.97	0.04^{s}	0.01^{s}
LV Mass Index	152.09 ± 30.34	129.68± 31.84	117.33 ± 28.33	0.12^{ns}	0.02^{s}

Data expressed as Mean ± SD
Data analyzed using unpaired t-test
ns = Not significant
s = Significant

Table-V:Comparison of post-operative echocardiographic variables at 1 month between two groups (N=60)

Echocardiographic variables	Group A	Group B	P-value
LVEDD	48.7 ± 5.03	49.5 ± 5.66	0.74^{ns}
LVESD	32 ± 4.24	35 ± 2.49	0.07^{ns}
LVEF	57.18 ± 9.55	49.91± 8.61	0.09^{ns}
IVST	10.3 ± 1.7	11.5 ± 1.08	0.08^{ns}
PWT	9.6 ± 0.84	10.9± 0.88	0.003^{s}
LV Mass Index	108.54 ± 31.13	129.68± 31.84	0.15^{ns}

Data expressed as Mean ± SD
Data analyzed using unpaired t-test
ns = Not significant
s = Significant

Table-VI: Comparison of post-operative echocardiographic variables at 3 month between two groups (N=60)

Echocardiographic variables	Group A	Group B	P-value
LVEDD	47.7 ± 4.37	48.2 ± 5.71	0.83^{ns}
LVESD	29.6 ± 3.53	33.7 ± 2.5	0.008^{s}
LVEF	62.04± 7.89	50.99 ± 8.77	0.008^{s}
IVST	9.2 ± 1.03	11 ± 0.82	0.0004^{s}
PWT	8.9 ± 0.99	10.5 ± 0.97	0.002^{s}
LV Mass Index	91.76 ± 23.24	117.33 ± 28.33	0.04^{s}

Data expressed as Mean ± SD
Data analyzed using unpaired t-test
ns = Not significant
s = Significant

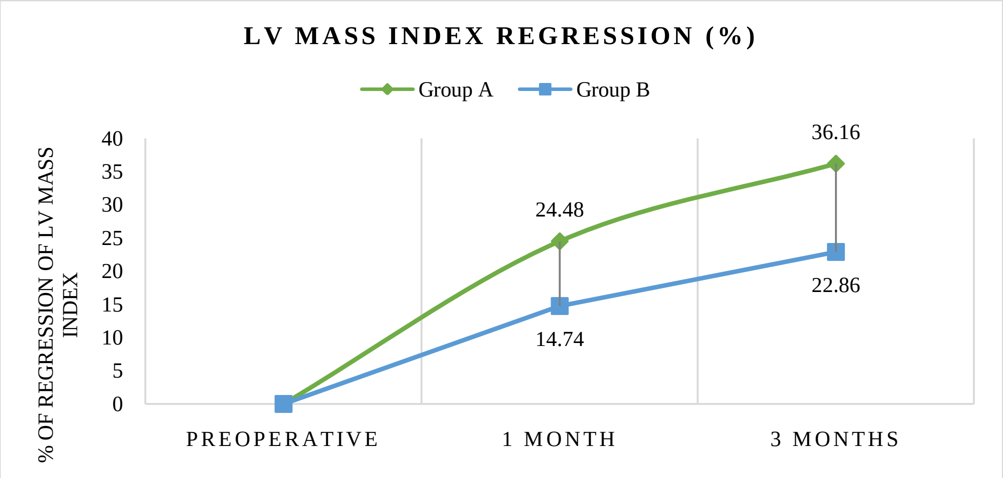

Figure-1: Comparison of regression of LV Mass Index after surgery between two groups

Table-VII: Comparison of regression of LV Mass Index after surgery between two groups (N=60)

	Pre-operative (gm/m²)	1 month (gm/m²)	Regression at 1 month (%)	3 months gm/m²	Regression at 3 months (%)
Group-A	143.74	108.54	24.48	91.76	36.16
Group-B	152.09	129.68	14.74	117.33	22.86

V. Discussion

This current study was conducted at Department of Cardiac Surgery, Bangabandhu Sheikh Mujib Medical University, Dhaka from August, 2020 to July, 2022. Total 60 patients were enrolled in this study who underwent aortic valve replacement for isolated aortic valve disease. These patients were assigned into two groups. Each group consisted equal numbers of patients (30 in each group). Group-A patients received metallic valve whereas Group-B patients received tissue valve.In this study the Mean ± SD age of Group-A was 55.1 ± 10.96 years and in Group-B was 61.7 ± 16.91 years. The means of age of two groups were compared statistically and revealed no significant difference (p>0.05). In a study on mechanical or biologic prosthesis for aortic and mitral valve replacement conducted over a period of 1996-2013 included 9942 patients who underwent AVR. In this study Goldstone BA et al.[15] found that, 6097 patient received mechanical aortic valve with a mean age of 56.3 ± 5.5 years and 3845 patients received biologic aortic valve with mean age of 57.4± 5.3 years. They found no statistical difference in mean age. Their finding is similar to this study. In this study majority of the patients were male in both groups. Out of the 60 patients, 44 (73.3%) patients were male. In Group-A 21(70%) and in Group-B 23(76.7%) patients were male. Gender comparison between two groups was not statistically significant (p>0.05). In the study of De Paulis R et al.[16], females needed most of the tissue valves compared to metallic valve. However, sex distribution was not statistically significant among the recipients. This is similar to the study findings. This current study observed, majority of the patient of both groups belongs to normal BMI groups. In Group-A 24(80%) and in Group-B 20(66.7%) patients had normal BMI. (<24.9) Both groups were compared statistically and revealed no significant difference (p>0.05).McClure RS et al.[17]found that median BMI of aortic valve recipients was 29.2 and 28.9 in tissue and metallic valve groups respectively. However, they observed no significant statistical difference. In our study, mean ± SD of BSA of both groups were 1.63 ± 0.14 m^2 and 1.64 ± 0.15 m^2 in Group-A and Group-B subjects respectively. Comparison of mean BSA of both groups was statistically not significant (p>0.05). De Paulis R et al.[16] reported mean BSA of their study subjects was 1.69 ± 0.1 and 1.70 ± 0.1 m^2 in tissue and metallic valve recipients respectively. There was no significant difference. This is similar to the study finding. In the present study, pre-operative mean values of LVEDD, LVESD, LVEF, IVST, PWT and LV mass index were 51.5 ± 5.06, 35.7 ± 5.5, 52.11 ± 12.09, 11.8 ± 1.93, 11.1 ± 1.1 and 143.74 ± 39.78 in Group-A and 50.6 ± 4.99, 37.1 ± 2.6, 46.51 ± 6.59, 12.7 ± 1.16, 11.9 ± 1.2 and 152.09 ± 30.34 in Group-B respectively. Means were compared statistically between two groups and revealed no significant difference (p>0.05). Jin XY et al.[18]found that mean values of LVEDD, LVESD, IVST, PWT and LV mass index were 52 ± 8, 40 ± 8, 13 ± 2, 15 ± 3 and 215 ± 100 in metallic valve group whereas 52 ± 10, 41 ± 10, 14 ± 3, 16 ± 3 and 225 ± 105 in tissue valve group respectively. They observed no significant difference in mean values of different echocardiographic variables. This is similar to the study result. In Italy, De Paulis R et al.[16] studied LVH regression after AVR with different valve substitute compared metallic versus tissue valve recipients (10 on each group). Their preoperative mean values of LVEDD index, LVESD index, LVEF, IVST, PWT and LV mass index were 29.4 ± 2, 18.4 ± 1.9, 65 ± 7, 15 ± 2.1, 13.4 ± 2.5 and 216 ± 36 in metallic valve group whereas 28.3 ± 4.7, 18.4 ± 5.7, 63 ± 12, 14.6 ± 2.7, 12.2 ± 1.3 and 185 ± 56 in tissue valve group respectively. They observed no significant difference in mean values of different echocardiographic variables. This is comparable to the study result. Post operative data of both groups of our study were recorded at 1month and 3months after surgery. Group-A patients showed significant reduction of PWT (from 11.1 ± 1.1 to 9.6 ± 0.84) and LV mass index (from 143.74 ± 39.78 to 108.54 ± 31.13) (p<0.05). Group-B patients showed significant reduction of IVST (from 12.7 ± 1.16 to 11.5 ± 1.08) and PWT (from 11.9 ± 1.2 to 10.9 ± 0.88) (p<0.05). While comparing both groups, this study observed, both the groups shared significant regression of PWT at 1month follow-up. However, during this period, only Group-A subjects had significant reduction of LV mass. This study also observed that, at 1 month period, PWT in Group-A patients was 9.6 ± 0.84 and in group-B patient was 10.9± 0.88. Difference in regression of PWT between two groups was statistically significant (p<0.05), whereas, despite improvement in LV mass index in Group-A and IVST in Group-B, the changes between the groups was not statistically significant (p>0.05). In the study of Jin XY et al.[18], irrespective of valve substitute, all the patients showed significant reduction in PWT and LV mass index compared to pre-AVR values. Their findings are similar to our study. Therefore, it is evident that after AVR the early changes occur especially in regression of PWT, IVST and LV mass index. However, this study observed regression in PWT in both groups and regression of LV mass index in Group-A and IVST in Group-B whereas Jin XY and colleagues observed regression in PWT and LV mass index in both groups.[18]Interestingly, Jin XY et al.[18] observed superior regression of IVST, PWT and LV mass index in tissue valve recipients compared to metallic valve recipients which is quite opposite to what this study observed. At 3 months follow-up, the study observed significant improvement of LVESD (from 35.7 ± 5.5 to 29.6 ± 3.53), LVEF (from 52.11 ± 12.09 to 62.04± 7.89), IVST (from 11.8 ± 1.93 to 9.2 ± 1.03), PWT (from 11.1 ± 1.1 to 8.9 ± 0.99) and LV mass index (from 143.74 ± 39.78 to 91.76 ± 23.24) (p<0.05) in Group-A subjects. Group-B subjects showed significant regression of IVST (from 12.7 ± 1.16 to 11 ± 0.82), LVESD (from37.1± 2.6 to 33.7± 2.5) PWT (from 11.9 ± 1.2 to 10.5 ± 0.97) and LV mass index (from 152.09 ± 30.34 to 117.33 ± 28.33) (p<0.05). Therefore, At 3 months follow-up, the study observed, both groups showed significant improvement in IVST,LVESD, PWT and LV mass index compared to pre-operative values (p<0.05). In addition to this, Group-A also showed significant improvement in LVEF (p<0.05) in respect to pre-operative values. Regression of LVH after AVR was studied by De Paulis R et al.[16]. They observed, after 1 year, changes in echocardiographic value of tissue valve recipients were not statistically significant while metallic valve recipients showed significant reduction in LVEDD index, IVS, PWT and LV mass index compared to pre-AVR values. Comparing these two groups, none of the parameters were significant at 1-year follow-up. They concluded due to small number of study subjects their inference regarding LV mass regression between two groups was inconclusive.From these studies it is apparent that, LVEDD, IVS, PWT and LV mass index regression is better observed in metallic valve recipients compared to tissue valve recipient in early period. However, the changes may become not significant in long term follow-up.

Limitations of the study

There are some limitations of this study. The number of patients was limited because this study enrolled the patients suffering from isolated aortic valve disease in a short period of time. The small sample size

(60) and the short study period limit the quality of the result that could be obtained. This could have some influences on statistical power and variables. Here echocardiography was done to see LV morphology and functional changes. Echocardiographic measurements were depended on quality of image. No histological examination was performed in this study. Furthermore, we followed up patients for up to three months after aortic valve replacement and myocardial remodeling might need long term observation.

VI. Conclusion and recommendations

This study observed superior left ventricular mass regression after aortic valve replacement with metallic valve in comparison to tissue valve.After initial beneficial effects imparted by AVR in severe AS patients, there are, as expected, marked improvements in LV reverse remodeling. surgically induced benefits to LV structure and function are durable and unexpectedly express continued, albeit markedly incomplete improvement through post-AVR concordant with sustained improved clinical status. Further multicenter study with larger sample size and extended study period is recommended.

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