Section: Pathology

Original Research Article

COMPARATIVE HISTOMORPHOLOGICAL ANALYSIS OF AORTIC VALVE SPECIMENS IN RHEUMATIC AND NON-RHEUMATIC AORTIC STENOSIS: A DESCRIPTIVE STUDY FROM A TERTIARY CARE CENTRE

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ABSTRACT

Background: In the beginning of 20th century, Rheumatic heart disease was the leading cause of heart valve illness, involving most commonly mitral valve, second being aortic valve. Degenerative non-Rheumatic aortic stenosis has become the most important cause of aortic valve obstruction. **Objective:** To compare the histomorphological features of aortic valve specimens in Rheumatic and non-Rheumatic aortic stenosis at Department of Pathology, Government Medical College, Kottayam.

Materials and Methods: It was a Descriptive study done among 140 aortic valve samples (70 cases of rheumatic aortic stenosis and 70 cases of non rheumatic aortic stenosis), taken from patients admitted at cardiothoracic and vascular surgery department, Govt. medical college, Kottayam. After consent from IRB, specimen of aortic valve were collected from Cardiothoracic department, Government medical college, Kottayam. Valves were fixed in 10%formalin. Representative samples were taken from each valve. One section was taken from each cusp and stained with H and E stain and studied under light microscope. All patients admitted for surgery for Rheumatic and non-Rheumatic aortic stenosis at Department of Cardiothoracic and Vascular surgery, Government Medical College, Kottayam were included in the study. Specimens where sample is not adequate were excluded from the study.

Results: In our study, all valves studied for RAS were tricuspid, however in NRAS both tricuspid and bicuspid were involved. Majority of the valves of both rheumatic and non rheumatic aortic stenosis showed evidence of diffuse microcalcification. There was no evidence of hemorrhage among valves with RAS and NRAS.

Conclusion: On comparing the histomorphological features of Rheumatic and non-rheumatic aortic stenosis valves, showed almost similar findings except for type of valve involvement.

Keywords: Rheumatic Aortic Stenosis, Non-Rheumatic Aortic Stenosis, Aortic Valve Histomorphology, Microcalcification, Mononuclear Infiltration

INTRODUCTION

Aortic stenosis is a common valvular disorder mainly seen in elderly population it can be due to various causes which include congenital, calcific and rheumatic disease. Patients can develop chest pain, heart failure and syncope. It can lead to left ventricular outflow obstruction.[1] Aortic stenosis is the second most common valvular disease in the western world after mitral regurgitation and affects 2% of the population of age between 65 and 75 years and 6 % those older than 75 years and is often associated with other valvular disease. Degenerative etiology comprises the majority of cases nowadays,

but when associated with other heart diseases rheumatic heart disease must be considered.[2] Vascular calcification, like coronary and aortic calcification, is a significant feature of vascular pathology, since this lesion is associated with cardiovascular disease. Statins are potent serum cholesterol reducing drugs and help in reducing the risk of cardiovascular diseases. Besides reducing serum cholesterol levels, statins are shown to decrease the rate of coronary artery calcification by unidentified mechanisms. Though some observational studies show that statins decrease rate of calcific aortic valve stenosis, few recent prospective studies reveal that statins are not effective against the progression of calcific aortic stenosis.[3,4]

Aortic stenosis can be treated medically or surgically based on the severity and /or symptoms. Surgical treatment may consist of repair of valve leaflets or replacement of the valve. Surgery can either be an open repair or minimally invasive based on the individual cases.[5] Open surgical valve replacement is the treatment of choice for aortic stenosis. In this procedure, the valve leaflets are repaired or the damaged valve is removed and replaced with a new valve. For aortic stenosis patients who are not candidates for open heart surgery, minimally invasive transcatheter aortic valve replacement (TAVR) can be done.[6]

The present study is a small descriptive study focusing mainly on possible differences in histopathology in valves of rheumatic and non-rheumatic aortic stenosis. The histomorphological differences expected based on previous studies are: increased lymphocytic infiltration in valves with non-rheumatic aortic stenosis when compared with rheumatic aortic stenosis, calcification to be more localized to the base of cusps in non-rheumatic aortic stenosis and diffuse calcification in valves with rheumatic aortic stenosis. Grading of microcalcification, inflammatory infiltration and haemorrhage were done based on previous studies.

MATERIALS AND METHODS

It was a Descriptive study done for a period of Eighteen months after the IRB approval date in the Department of Pathology, Government Medical College, Kottayam.

Inclusion Criteria

All patients admitted for surgery for Rheumatic and non-Rheumatic aortic stenosis at Department of Cardiothoracic and Vascular surgery, Government Medical College, Kottayam.

Exclusion Criteria

Specimens where sample is not adequate.

Sample size

According to study by Lars Wallby et al[7] on inflammatory characteristics of stenotic aortic valves, a comparison between rheumatic and non-rheumatic aortic stenosis and T lymphocyte were seen in 80% of rheumatic aortic stenosis and 90% of non rheumatic aortic stenosis valves. With this, sample size is

calculated by the formula,

$N = (Z\alpha + Z\beta)^2 PQ \times 2/D^2$

$Z\alpha = 1.96$ for α at 5% level of significance

$Z\beta = 0.84$ at 80% power

$P = (P1 + P2)/2$

$Q = 100 - P$

$P1 = 80$; $P2 = 90$

$P = (P1 + P2)/2 = (80+90)/2 = 85$

$Q = 100 - P = 100 - 85 = 15$

D=Precision

D = 20% of P =17

Thus, $N = (1.96 + 0.84)^2 \times 85 \times 15 \times 2/ 17 \times 17 = 70$ in each group

Thus the sample size to be calculated was 70 cases of rheumatic aortic stenosis and 70 cases of non rheumatic aortic stenosis. Due to covid issues, sample size could not be attained .12 cases of rheumatic aortic stenosis and 28 cases of non rheumatic aortic stenosis were obtained and the present study is conducted on this sample size of total size 40 cases. Aortic valve specimens were used as study tool.

After consent from IRB, specimen of aortic valve are collected from Cardiothoracic department, Government medical college, Kottayam. Valves are fixed in 10%formalin. Representative samples are taken from each valve. One section is taken from each cusp and stained with H and E stain and studied under light microscope. Calcification is estimated by microscopic analysis of specimen by staining with Von Kossa stain. Grossly calcified valves are decalcified in 10%formic acid solution for 24hrs, processed, cut and stained using H and E and Von Kossa stains and examined under microscope. Localisation of calcification is also studied

Extent of valvular microcalcification is graded as:

0 = absent

Trace = deposits not clearly visible on low power

Mild= scattered loose deposits or dense focal deposits covering less than 2 high power field

Moderate= dense deposits in more than 2 and less than 6 high power field

Severe= dense deposits in 6 or more high power field

Degree of mononuclear infiltration is graded as described by Stratford et al into:

0= no inflammatory cells present

1+ =occasional scattered cells or one group of 20 cells in a cusp section

2+ =several groups of 20 cells or more in a cusp section

3+ =many group of more than 20cells or one group of 100 cells or more in a cusp section

Valves are evaluated for old haemorrhage using Perls stain and for fresh haemorrhage using H and E stain and both are graded as

0 =absent

Trace= hemosiderin deposits or fresh haemorrhage seen focally in one high power field

Mild= hemosiderin deposits or fresh haemorrhage seen in 2 high power field

Moderate= deposit seen in more than 2 and less than 6 high power field
Severe = deposits in 6 or more high power field.

Data management and analysis

Data collected is entered in the MS Excel spread sheet and is analysed at the end of the study using SPSS software (version 26). Mean for age, type of valve, microcalcification distribution, degree of mononuclear infiltration, grading for haemorrhage and microcalcification were studied.

RESULTS

Table 1: Age wise distribution between two groups

	Mean age	Standard deviation	Minimum age	Maximum age
Rheumatic aortic stenosis	46.17	15.561	20	64
Non rheumatic aortic stenosis	61.43	8.126	46	80

The mean age of patients with rheumatic aortic stenosis : 46.17+/-15.56years. The minimum age is 20 years, and the maximum age is 64 years. The mean age of patients with non-rheumatic aortic stenosis : 61.43+/-8.12 years. The minimum age is 46 years , and the maximum age is 80 years. [Table 1]

Among the total valves studied(N=40), 2(7.1%) out of 28 non rheumatic aortic stenosis valves were bicuspid. All the rheumatic aortic stenosis valves studied(n=12), were tricuspid (100%). Both the bicuspid valves were seen in female patients. [Figure 1]

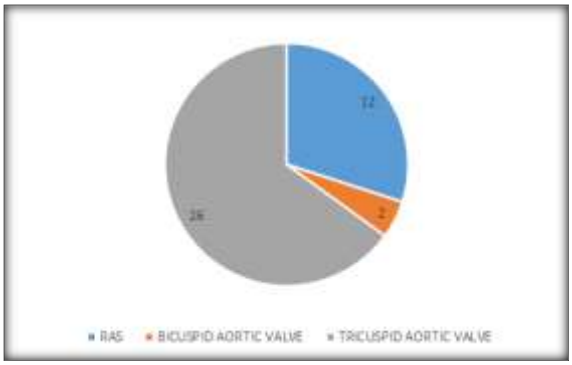

Figure 1: Type of valve involved in RAS and NRAS (N=40)

Table 2: Site and Distribution of Microcalcification in RAS and NRAS

Site	RAS	NRAS
ABSENT	25% (3/12)	10.7% (3/28)
MIDDLE	0	7.1% (2/28)
BASE	8.3% (1/12)	7.1% (2/28)
MIDDLE + BASE	16.7% (2/12)	17.9% (5/28)
DIFFUSE(6)	50% (6/12)	53.6% (15/28)
TIP+MIDDLE	0	3.6% (1/28)

3 out of 12 valves with rheumatic aortic stenosis showed no evidence of microcalcification (25%). 1 out of 12 valves of rheumatic aortic stenosis showed calcification at the base of valve (8.3%). 2 out of 12 valves with rheumatic aortic stenosis showed evidence of calcification restricted to the middle part and base (16.7%). 6 out of 12 valves with rheumatic aortic stenosis showed no definite site predilection and there was diffuse calcification along the tip, middle piece and base (50%). Thus among the valves with rheumatic aortic stenosis , 75% of valves showed evidence of microcalcification (9/12 valves). Among the valves with non rheumatic aortic stenosis , 3 valves showed no evidence of microcalcification (10.7%)2 out of 28 valves of patients with non rheumatic aortic stenosis showed evidence of microcalcification at the middle part of the valve(7.1%).2 out of 28 valves of non rheumatic aortic stenosis patients showed evidence of microcalcification at the base of the valve(7.1%)1 out of 28 valves showed evidence of microcalcification at the tip and middle piece of the valve.(3.6%)5 out of 28 valves showed evidence of microcalcifcation confined to the middle piece and base of the valve cusp(17.9%).15 out of 28 valves(53.6%) showed diffuse calcification involving the valve tip, middle piece and base. Among the non rheumatic aortic stenosis valves, more than 50 percent valves showed diffuse calcification.

Table 3: Degree of mononuclear infiltration(n=40) (in percentage)

GRADING	RAS	NRAS
0	25% (3/12)	25% (7/28)
1+	66.7% (8/12)	60.7% (17/28)
2+	8.3% (1/12)	14.3% (4/28)
3+	0	0

Among valves studied with rheumatic aortic stenosis :3/12 valves (i.e.,25%) showed no evidence of infiltration by any inflammatory cells. 8/12(66.7%)valves showed occasional scattered cells or one group of 20 cells in a cusp section. 1/12(8.3%) valves showed several groups of 20 cells or more in a cusp section. None of the valves showed many groups of more than 20 cells or one group of 100 cells or more in a cusp section. Among the valves studied with non rheumatic aortic stenosis. 7/28(25%) valves showed no evidence of any infiltration.17/28(60.7%) valves showed occasional scattered cells or one group of 20 cells in a cusp section. 4/28(14.3%) valves showed several groups of 20 cells or more in a cusp section.

-None of the valves showed many groups of more than 20 cells or one group of 100 cells or more in a cusp section.

Table 4: Grading of Microcalcification(n=40)

SITE	NRAS	RAS
ABSENT	10.7% (3/28)	25% (3/12)
TRACE	7.1% (2/28)	0
MILD	35.7% (10/28)	41.7% (5/12)
MODERATE	25% (7/28)	25% (3/12)
SEVERE	21.4% (6/28)	8.3% (1/12)

3 out of 28 valves (i.e., 10.7%) of NRAS showed no evidence of microcalcification. 2 out of 28 valves with non rheumatic aortic stenosis showed evidence of trace calcification.10 out of 28 valves (i.e.,35.7%) showed evidence of mild calcification.7 out of 28 valves (i.e., 25%) showed evidence of moderate calcification.6 out of 28 valves (i.e. 21.4%) studied showed evidence of severe calcification. Among the valves with non rheumatic aortic stenosis, majority of the valves showed evidence of mild calcification. 3 out of 12 valves with rheumatic aortic stenosis were not having any evidence of calcification. None of the valves showed any evidence of trace calcification.5 out of 12 valves (i.e., 41.7%) showed evidence of mild calcification.3 out of 12 valves (i.e., 25%) showed evidence of moderate calcification.1 out of 12 valves with rheumatic aortic stenosis showed evidence of severe calcification. Among the 12 valves studied, majority of the valves showed evidence of mild calcification.

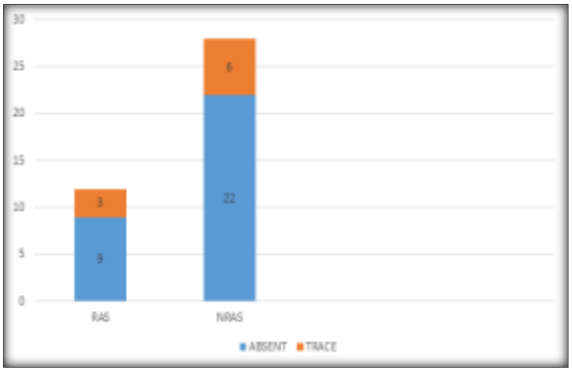

Figure 2: Grading for Haemorrhage(n=40)

Among valves with rheumatic aortic stenosis. Majority of valves [9/12(75%)] showed no evidence of haemorrhage. 3/12(25%) valves showed evidence of trace haemorrhage. Among valves with non rheumatic aortic stenosis, Majority of valves [22/28(78.6%)] showed no evidence of haemorrhage. 6/28(21.4%) valves showed evidence of trace haemorrhage. None of the valves among rheumatic aortic stenosis and non rheumatic aortic stenosis showed any evidence of mild, moderate or severe haemorrhage.

DISCUSSION

According to the literature, macroscopic features of rheumatic aortic stenosis are thickened and fused cusps dominated by fibrosis. In a study by Waller et al,[8] aortic valve was the most frequently excised native cardiac valve. Among them,91% were stenotic (with or without regurgitation) and 9% pure regurgitant. In more than 95% of the stenotic aortic valves, etiology belongs to one of the three types: congenital, degenerative and rheumatic. Other rare causes were active infective endocarditis, homozygous type 2 hyperlipoproteinemia and systemic lupus erythematosus. There are multiple causes of pure aortic regurgitation but they can be separated into diseases affecting valve with normal aorta (eg., infective endocarditis, congenital bicuspid), diseases affecting the walls of aorta but with normal valve (eg., syphilis, Marfans dissection), disease affecting both aorta and valve(eg., ankylosing spondylitis), and disease affecting neither aorta nor valve (eg., ventricular septal defect, systemic hypertension).

In a study conducted by Wallby et al,[7] ratio of rheumatic (26%) versus non rheumatic aortic valves was greater than previously reported by Dare et al,[9] (9%) and in the same range as given by Passik et al,[10] (20%). In this study, among the total 39 valves studied with aortic stenosis.[10] patients were considered to have aortic valve disease of rheumatic origin based on gross valvular pathology of thickened and fused cusps. Three of them had history of rheumatic fever and had additional rheumatic diseases of mitral valve. Other 7 patients did not have history of rheumatic fever and were devoid of echocardiographic signs of mitral valve stenosis. Rest of the 29 patients had valves diagnosed with non rheumatic aortic stenosis. The mean age of patients with rheumatic aortic stenosis was observed to be 64+/-7 years.Among the non rheumatic aortic

stenosis cases , 12 valves were bicuspid and rest of the 17 valves were tricuspid. The mean age for bicuspid valves were 67+/-8 years and for tricuspid valves were 71+/-7 years. In our study, out of the 40 valves studied, 12 valves were rheumatic aortic stenosis and 28 valves were non rheumatic aortic stenosis. The mean age of patients with rheumatic aortic stenosis in our study was 46.17+/- 15.56 years. The minimum age was 20 years and the maximum age was 64 years. The mean age of patients with non rheumatic aortic stenosis was 61.43 +/- 8.12 years. The minimum age was 46 years and the maximum age was 80 years.

In the same study by Wallby et al,[7] neovascularization was noticed in 12/29 (41%) valves with non rheumatic aortic stenosis and 3/10(30%) valves with rheumatic aortic stenosis. In a study conducted by Goffin et al,[11] 63 surgical patients were chosen with verified history of rheumatic fever and whose aortic valve was removed either singly or along with other valves. In 22 cases, the histological examination of aortic valves showed functional lesions. In 19 valves, there was destruction of architecture with scarring, organic lesions of inflammatory origin and hypertrophic vessels. They used fibrotic scar tissue and neovascularization as the histopathological markers for rheumatic valve disease. One – third of cases fulfilled both criteria, one third revealed only functional changes while remaining one – third fulfilled only one criteria and was thus difficult to interpret. It was concluded that there is no significant correlation between anatomical aspects of the aortic valve deformity and the presence of histologically proven organic lesions of inflammatory origin, thus supporting the opinion that rheumatic carditis mainly involves mitral valve. In our study, neovascularization was seen in 1/12(8.3%) valves with rheumatic aortic stenosis. Majority of the valves (11/12i.e., 91.7%) with rheumatic aortic stenosis showed no evidence of neovascularisation. Among the valves with non rheumatic aortic stenosis, all the 28 valves showed no evidence of neovascularization.

In our study, microcalcification was present in majority of the valves studied and was a common histopathological feature for both rheumatic and non rheumatic aortic stenosis. Among the total 40 valves studied, microcalcification was seen in 34 cases. Among the valves with rheumatic aortic stenosis, calcification was seen in 9/12(75%) valves. Among the valves with non rheumatic aortic stenosis, calcification was seen in 25/28(89.3%) cases. Even though microcalcification was seen more in patients with non rheumatic aortic stenosis, it was also seen in majority of cases with rheumatic aortic stenosis thus not contributing as a histopathological feature to distinguish between them, thus being similar to the study by Wallby et al,[7] 1/12(8.3%) valves with rheumatic aortic stenosis showed evidence of microcalcification localized to the base of cusp. Majority of the valves with rheumatic aortic stenosis (i.e.,50%) showed no definite site predilection and had diffuse calcification. Among the valves with non rheumatic aortic stenosis, 15/28(53.6%) showed evidence of diffuse calcification. Thus in our study, majority of the valves of both rheumatic and non rheumatic aortic stenosis showed evidence of diffuse microcalcification.

The difference in calcification between non rheumatic aortic stenosis with bicuspid valves and tricuspid valves is previously described by Isner et al.[12] They investigated 30 heavily calcified aortic valves. They found nodular calcific deposits in 11/16 cases of non rheumatic aortic stenosis with tricuspid valves and diffuse calcification in 14/14 cases of non rheumatic aortic stenosis with bicuspid valves. In our study, there were 2 bicuspid valves among the 28 valves with non rheumatic aortic stenosis. Microcalcification was present in both bicuspid valves.

CONCLUSION

Among the valves studied with rheumatic and non rheumatic aortic stenosis, the mean age of patients with rheumatic aortic stenosis was 46.17+/-15.56years and for non-rheumatic aortic stenosis was 61.43+/-8.12 years. Microcalcification was a major histopathological feature of both rheumatic and non rheumatic aortic stenosis, even though some of the cases showed site predilection , it cannot be taken as a histomorphological criteria for diagnosing rheumatic or non rheumatic aortic stenosis. The grading for mononuclear infiltration was given as 1+ for majority of cases with rheumatic and non rheumatic aortic stenosis. There was no evidence of hemorrhage among valves with RAS and NRAS.

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