Section: Pathology

Original Research Article

A COMPARATIVE STUDY FROM A TERTIARY CARE CENTER COMPARING THE HISTOMORPHOLOGICAL CHARACTERISTICS OF AORTIC VALVE SPECIMENS IN RHEUMATIC AND NON-RHEUMATIC AORTIC STENOSIS

Jisha Raj[1], Anamika Devarajan[2], Anseena Kanjirathinkal Muhammed[3], Athira Sarada[4]

[1]*Assistant Professor, Department of Pathology, Mount Zion Medical College, Chayalode, Adoor, India.*
[2]*Assistant Professor, Department of Pathology, Mount Zion Medical College, Chayalode, Adoor, India.*
[3]*Assistant Professor, Department of Pathology, Mount Zion medical College, Chayalode, Adoor, India.*
[4]*Assistant Professor, Department of Pathology, Mount Zion Medical College, Chayalode, Adoor, India.*

Received *: 29/08/2025*
Received in revised form *: 12/10/2025*
Accepted *: 30/10/2025*

Corresponding Author:
Dr. Athira. S,
Assistant Professor, Department of Pathology, Mount zion Medical College, Chayalode, Adoor, India.
Email:drathira6850@gmail.com

DOI: 10.70034/ijmedph.2025.4.176

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (4); 983-988

ABSTRACT

Background: Aortic stenosis (AS) is a significant valvular heart disease that can arise from either rheumatic or non-rheumatic etiologies, each exhibiting distinct histomorphological features. Rheumatic aortic stenosis (RAS) is commonly associated with chronic inflammation and fibrosis resulting from rheumatic fever, while non-rheumatic aortic stenosis (NRAS) is primarily attributed to age-related degenerative changes or congenital anomalies. Identifying these histological differences is crucial for accurate diagnosis and management. **Objective:** The study aimed to compare the histomorphological characteristics of aortic valve specimens in patients with rheumatic and non-rheumatic aortic stenosis to delineate distinguishing pathological features.

Materials and Methods: A descriptive study was conducted over eighteen months in the Department of Pathology, Government Medical College, Kottayam. A total of 40 aortic valve specimens were obtained, including 12 from RAS and 28 from NRAS patients undergoing valve replacement surgery. The specimens were fixed in 10% formalin, sectioned, stained with Hematoxylin & Eosin and Von Kossa stains, and examined under a light microscope. Histomorphological parameters like fibrosis, calcification, mononuclear infiltration, and neovascularization were compared between the two groups. Statistical analysis was performed using SPSS software (version 26).

Results: Among RAS patients, 83.3% were females, while NRAS had an equal gender distribution. Predominant mononuclear infiltration in RAS was lymphocytes (58.3%), while NRAS showed a higher infiltration of lymphocytes (71.4%) with minimal plasma cells. Fibrosis was observed in 75% of RAS and 50% of NRAS cases. There was no association of neovascularisation in either group.

Conclusion: The study highlights distinct histomorphological differences between rheumatic and non-rheumatic aortic stenosis. Rheumatic aortic stenosis is predominantly characterized by commissural fusion, dense fibrosis, and inflammatory infiltration, while non-rheumatic aortic stenosis exhibits diffuse calcification and minimal inflammatory infiltration. Identifying these features is crucial for accurate diagnosis, prognostication, and tailoring surgical management in patients with aortic stenosis.

Keywords: Aortic stenosis, Rheumatic aortic stenosis, Non-rheumatic aortic stenosis, Histomorphological analysis, Calcification, Fibrosis, Mononuclear infiltration, Valve pathology, Tertiary care center.

INTRODUCTION

Aortic stenosis (AS) is a significant valvular heart disease characterized by the narrowing of the aortic valve opening, which obstructs blood flow from the left ventricle to the aorta. It can result from a variety of etiologies, prominently including rheumatic and non-rheumatic causes. Rheumatic aortic stenosis typically arises from chronic inflammatory changes secondary to rheumatic fever, while non-rheumatic aortic stenosis primarily results from age-related degenerative calcification, bicuspid aortic valves, or congenital anomalies.[1,2]

The distinction between rheumatic and non-rheumatic aortic stenosis holds substantial clinical relevance due to differences in pathogenesis, progression, and therapeutic approach. Rheumatic heart disease (RHD) predominantly affects younger individuals in developing countries, whereas degenerative calcific aortic stenosis is more prevalent in the elderly population worldwide. Understanding the histomorphological differences between these two conditions is critical for accurate diagnosis, prognostication, and management, as well as for advancing future research in cardiovascular pathology.[3,4]

Rheumatic Aortic Stenosis

Rheumatic aortic stenosis is a consequence of chronic inflammation following acute rheumatic fever (ARF), which is primarily triggered by Group A Streptococcus infection. The immune response initiated during ARF leads to cross-reactivity between streptococcal antigens and cardiac tissues, resulting in progressive valvular damage. The inflammatory process leads to fibrosis, commissural fusion, leaflet thickening, and eventual calcification of the aortic valve, contributing to valve stenosis.

Histologically, rheumatic aortic valves often show

- **Inflammatory infiltration:** Presence of mononuclear cells and lymphocytes.
- **Fibrosis:** Dense collagen like deposition and thickened leaflets.
- **Commissural fusion:** Fusion of valve leaflets, leading to narrowed valve orifice.
- **Calcification:** Dystrophic calcification in later stages of the disease.

Studies indicate that the burden of rheumatic aortic stenosis remains high in low- and middle-income countries due to delayed diagnosis and limited access to preventive strategies, such as secondary prophylaxis with antibiotics. Identifying distinct histological features in these patients can enhance diagnostic accuracy and inform surgical management strategies.[5,6]

Non-Rheumatic Aortic Stenosis

Non-rheumatic aortic stenosis primarily occurs due to age-related degenerative changes, congenital anomalies like bicuspid aortic valve, or chronic systemic conditions such as chronic kidney disease, diabetes, and hypertension. Age-related aortic stenosis is marked by progressive calcification, which begins at the base of the cusps and extends towards the leaflet body. This process is driven by endothelial dysfunction, lipid infiltration, and chronic inflammation, leading to:

- **Calcific Nodules:** Prominent dystrophic calcification without commissural fusion.
- **Fibrosis:** Sclerotic changes within the valve leaflets.
- **Neovascularization:** Formation of small blood vessels within the valve tissue.
- **Myxoid Degeneration:** Focal myxomatous changes in the valve leaflets.

Bicuspid aortic valve (BAV), a common congenital abnormality, predisposes individuals to early-onset aortic stenosis due to abnormal hemodynamic stress and increased calcification propensity. Unlike rheumatic aortic stenosis, non-rheumatic aortic stenosis rarely demonstrates commissural fusion or dense inflammatory infiltration, making histological comparison crucial for accurate etiological diagnosis.[7]

This study aims to conduct a comparative histomorphological analysis of aortic valve specimens obtained from patients with rheumatic and non-rheumatic aortic stenosis in a tertiary care setting. By evaluating key microscopic features such as fibrosis, calcification, inflammatory infiltration, neovascularization, and valve thickening, the study endeavors to delineate distinguishing pathological patterns that may assist in better understanding disease etiology and progression.

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 =$(1.96 + 0.84)^2$ x 85 x 15 x 2/ 17x17=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).Comparison between groups are performed using Fischer's exact test .The parameters analysed include gender distribution, fibrosis, microcalcification distribution, mononuclear infiltration, predominant type of mononuclear infiltration, association of neovascularisation among rheumatic and non rheumatic aortic stenosis. Association of fibrosis, microcalcification, and haemorrhage in rheumatic and non rheumatic aortic stenosis were also studied.

RESULTS

Table 1: Gender distribution of patients with rheumatic aortic stenosis and non-rheumatic aortic stenosis (n=40)

	GENDER	FREQUENCY	PERCENTAGE
RAS	MALE	2	16.7
	FEMALE	10	83.3
	TOTAL	12	100.0
NRAS	MALE	14	50.0
	FEMALE	14	50.0
	TOTAL	28	100.0

Among the rheumatic aortic stenosis patients (total 12 cases), more cases were females (83.3%) while in non rheumatic aortic stenosis patients (total 28 cases), no sex predilection was observed. Among the total cases studied (N=40) of patients with aortic stenosis, the predominant population were females (24 cases).

Table 2: Predominant type of Mononuclear Infiltration(n=40)

PREDOMINANT CELLS	RAS	NRAS
NO INFILTRATION	25% (3/12)	25% (7/28)
LYMPHOCYTES	58.3% (7/12)	71.4% (20/28)
PLASMA CELLS	16.7% (2/12)	3.6% (1/28)
MACROPHAGES	0	0

Among the valves with rheumatic aortic stenosis, 3 /12 valves showed no evidence of any infiltration. 7/12(58.3%) valves showed infiltration by predominantly lymphocytes. 2/12(16.7%) valves showed infiltration by predominantly plasma cells. None of the valves studied with rheumatic aortic stenosis showed any evidence of infiltration by macrophages. Among the valves with non rheumatic aortic stenosis, 7/28(25%) valves showed no

evidence of any infiltration. 20/28(71.4%) valves showed infiltration by predominantly lymphocytes. -1/28(3.6%) valves showed infiltration by predominantly plasma cells. None of the valves studied with non rheumatic aortic stenosis showed any evidence of infiltration by macrophages. Majority of the valves studied among both rheumatic aortic stenosis and non rheumatic aortic stenosis showed that predominant cells infiltrated were T lymphocytes.

Table 3: Association of fibrosis of valves in rheumatic aortic stenosis and non rheumatic aortic stenosis.(n = 40)

FIBROSIS	RAS	NRAS
PRESENT	9	14
ABSENT	3	14
TOTAL	12	28

According to Fischer's exact test : p – value:0.179 (>0.05). Since the p-value is >0.05 , there is no association of fibrosis in patients with rheumatic aortic stenosis and non rheumatic aortic stenosis.

Table 4: Association of microcalcification of valves in rheumatic aortic stenosis and non rheumatic aortic stenosis.(n = 40)

MICROCACIFICATION	RAS	NRAS
PRESENT	9	25
ABSENT	3	3
TOTAL	12	28

According to Fischer's exact test : p – value:0.341(>0.05). Since the p-value is >0.05, there is no association of microcalcification in patients with rheumatic aortic stenosis and non rheumatic aortic stenosis.

Table 5: Association of mononuclear infiltration of valves in rheumatic aortic stenosis and non rheumatic aortic stenosis. (n = 40)

INFILTRATION	RAS	NRAS
PRESENT	9	21
ABSENT	3	7
TOTAL	12	28

According to Fischer's exact test : p – value:1.000 (>0.05). Since the p-value is >0.05 , there is no association of mononuclear infiltration in patients with rheumatic aortic stenosis and non rheumatic aortic stenosis.

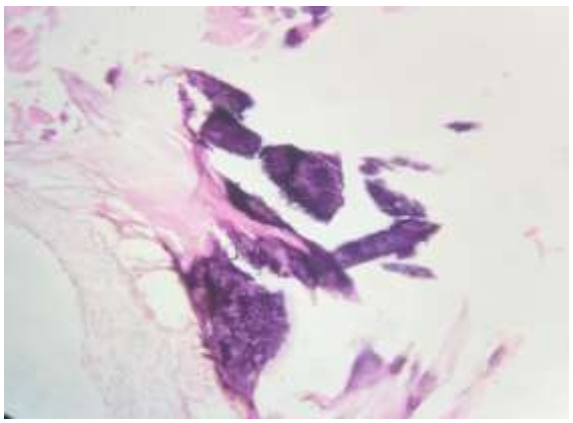

Figure 1: Microcalcification

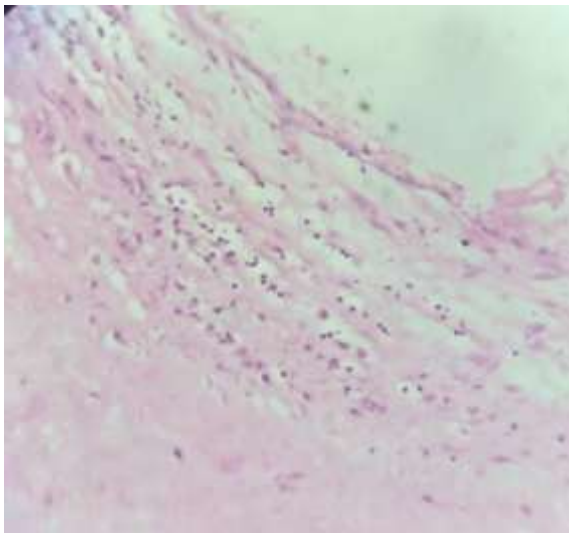

Figure 2: Mononuclear inflammatory infiltration

Table 6: Association of neovascularisation of valves in rheumatic aortic stenosis and non rheumatic aortic stenosis. (n = 40)

NEOVASCULARISATION	RAS	NRAS
PRESENT	1	0
ABSENT	11	28
TOTAL	12	28

According to Fischer's exact test: p – value:0.300 (>0.05). Since the p-value is >0.05, there is no

association of neovascularisation of valves in patients with rheumatic aortic stenosis and non rheumatic aortic stenosis.

DISCUSSION

Aortic valve specimens that are removed because of stenosis may show degenerative calcification of congenitally bicuspid valves, calcification of a normally three leaflet valve without commissural fusion (so called senile type degeneration),rheumatic changes or calcification of congenitally unicommisural valves. In some cases, it is difficult to differentiate congenital bicuspid aortic from a three leaflet valve that has calcified in a manner such that two of the leaflets fuse together. In such cases, leaflet sizes can be a helpful clue (the nonconjoined cusp in bicuspid valves is often disproportionately large). The presence of a usually midline raphe on the conjoined cusp can also help with this. According to literature, almost 50% patients with rheumatic heart disease donot have history suggestive of rheumatic fever.[7,8]

This study was conducted in patients who were admitted for surgery for rheumatic and non rheumatic aortic stenosis at Department of Cardiothoracic and Vascular Surgery, Government Medical college, Kottayam. This study was undertaken to know more about the histomorphology of both rheumatic and non rheumatic aortic stenosis and to analyse it for better understanding of stenotic disease of aortic valve. Total sample size obtained was 40, among which 12 cases were valves of patients with rheumatic aortic stenosis and 28 cases were valves of patients with non rheumatic aortic stenosis. The patients were divided as rheumatic aortic stenosis and non rheumatic aortic stenosis based on the echocardiography findings.

In transthoracic echocardiogram, calcific aortic stenosis is characterized by fibrocalcific masses on aortic side of leaflet that results in increased leaflet stiffness without commissural fusion, with a stellate shaped orifice in systole. Shadowing and rever beration limit image quality when obstruction is present, imaging shows a marked increase in echogenicity of the leaflets consistent with calcific disease and reduced systolic opening. Congenital bicuspid valve shows an elliptical shape of the open valve in systole.

Secondary calcification of a bicuspid valve can be difficult to distinguish from calcification of a trileaflet valve once stenosis becomes severe. M-mode recordings may help in identifying a bicuspid valve if an eccentric closure line is present but can be misleading in terms of degree of leaflet separation if the M-mode recording is taken through the base rather than the tips of the bowed leaflets. In rheumatic aortic stenosis, 2D and 3D imaging shows increased echogenicity along the leaflet edges, commissural fusion and systolic doming of the aortic leaflets.

In the study by Wallby et al,[7] 10 out of 39 valves revealed postinflammatory changes with severely distorted and fused cusp margins , resulting in central triangular orifice. These valves were considered as rheumatic aortic stenosis while the remaining 29 stenotic aortic valves were considered as non rheumatic aortic stenosis. In 1/10 rheumatic aortic stenosis valves, the valves were bicuspid while the remaining 9/10 valves were tricuspid. Among non rheumatic aortic stenosis valves, 12 valves were considered bicuspid and 17 valves as tricuspid. In our study, 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.

Calcification was only a minor feature according to Schoen and Sutton.[8] The histological findings are not specific and include architecture destruction, thickening due to collagen tissue
inflammatory cell infiltration, foci of calcification and sometimes ossification. In the study conducted by Wallby et al,[7] calcification was seen in both rheumatic aortic stenosis and non rheumatic aortic stenosis. However there was difference in localization of calcification. In non rheumatic tricuspid aortic stenosis , calcification was localized at the base of the cusps while in non rheumatic bicuspid aortic stenosis and rheumatic aortic stenosis valves ,there was diffuse calcification.

In another study by Wallby et al,[7] the objective was to compare non rheumatic tricuspid and bicuspid stenotic aortic valves for the presence and distribution of T lymphocytes. Valve specimens were obtained from 29 patients. T lymphocyte infiltration was seen in both tricuspid and bicuspid stenotic aortic valves but without any significant difference in its extent or localisation. They concluded that stenotic bicuspid aortic valves show same degree of T lymphocyte infiltration as degenerative tricuspid aortic valves.[9,10] They also concluded that inflammation need to be considered in the pathogenesis of acquired aortic stenosis, irrespective of the primary valve anomaly.

Wallby et al,[7] via a different study, found that the rheumatic aortic stenosis valves revealed somewhat lower degree of T lymphocyte infiltration when compared to non rheumatic aortic stenosis. Plasma cells were more commonly found in rheumatic aortic stenosis when compared to non rheumatic aortic stenosis, with lowest in non rheumatic aortic stenosis with tricuspid valves. All these figures suggested differences in the local inflammatory response although data was too limited to draw any conclusion.[11,12] In our study, mononuclear infiltration was seen in 9/12(75%) cases with rheumatic aortic stenosis and and 21/28 (75%) cases of non rheumatic aortic stenosis. Mononuclear infiltration was seen in both tricuspid and bicuspid valves. The predominant mononuclear cell infiltration in both rheumatic aortic stenosis and non rheumatic aortic stenosis were lymphocytes. 2/12 (16.7%) valves with rheumatic aortic stenosis and

1/28(3.6%) valves with non rheumatic aortic stenosis showed infiltration by plasma cells. None of the valves with both rheumatic aortic stenosis and non rheumatic aortic stenosis showed any evidence of infiltration by macrophages. While in the study by Wallby et al, macrophages were equally abundant in rheumatic aortic stenosis valves and valves with non-rheumatic aortic stenosis.[7]

CONCLUSION

Fibrosis was seen more in valves with rheumatic aortic stenosis and according to literature. Fibrosis is a major histomorphological feature of end stage rheumatic stenosis valves. Comparison of the histomorphological features of Rheumatic and non rheumatic aortic stenosis valves showed almost similar histomorphological features. A definite correlation could not be made out since the sample size could not be attained due to covid pandemics.

REFERENCES

1. Carabello BA. Introduction to aortic stenosis. Circulation research. 2013 Jul 5;113(2):179-85.
2. Suvarna SK, editor. Cardiac pathology: a guide to current practice. Springer Nature; 2019 Nov 11.
3. Butt HI, Shahbaz A, Nawaz H, Butt K. Comparative clinical characteristics of rheumatic heart disease patients undergoing surgical valve replacement. Cureus. 2019 Jun 12;11(6).
4. Remenyi B, Carapetis J, Wyber R, Taubert K, Mayosi BM. Position statement of the World Heart Federation on the prevention and control of rheumatic heart disease. Nature Reviews Cardiology. 2013 May;10(5):284-92.
5. Islam AM, Majumder AA. Rheumatic fever and rheumatic heart disease in Bangladesh: a review. Indian heart journal. 2016 Jan 1;68(1):88-98.
6. Carapetis JR, Beaton A, Cunningham MW, Guilherme L, Karthikeyan G, Mayosi BM, Sable C, Steer A, Wilson N, Wyber R, Zühlke L. Acute rheumatic fever and rheumatic heart disease. Nature reviews Disease primers. 2016 Jan 14;2(1):1-24.
7. Wallby L, Steffensen T, Jonasson L, Broqvist M. Inflammatory characteristics of stenotic aortic valves: a comparison between rheumatic and nonrheumatic aortic stenosis. Cardiology research and practice. 2013 Feb 14;2013
8. Schoen EJ, Sutton M. Contemporary pathologic considerations in valvular heart disease. Virmani R, Atkinson JB, Feuglio JJ. Cardiovascular pathology. Philadelhia, WB saunders Co. 1991:p334.
9. Okello E, Kakande B, Sebatta E, Kayima J, Kuteesa M, Mutatina B, Nyakoojo W, Lwabi P, Mondo CK, Odoi-Adome R, Juergen F. Socioeconomic and environmental risk factors among rheumatic heart disease patients in Uganda.
10. Omurzakova NA, Yamano Y, Saatova GM, Mirzakhanova MI, Shukurova SM, Kydyralieva RB, Jumagulova AS, Seisenbaev AS, Nishioka K, Nakajima T. High incidence of rheumatic fever and rheumatic heart disease in the republics of Central Asia. International journal of rheumatic diseases. 2009 Jul;12(2):79-83.
11. Alhaddad IA. Transcatheter Aortic Valve Replacement for Inoperable Severe Rheumatic Aortic Stenosis with Prior Mitral Valve Prosthesis. Journal of Structural Heart Disease. 2017 Aug;4(3):115-8.
12. Afifi A, Hosny H, Yacoub M. Rheumatic aortic valve disease—when and who to repair?. Annals of cardiothoracic surgery. 2019 May;8(3):383.