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]

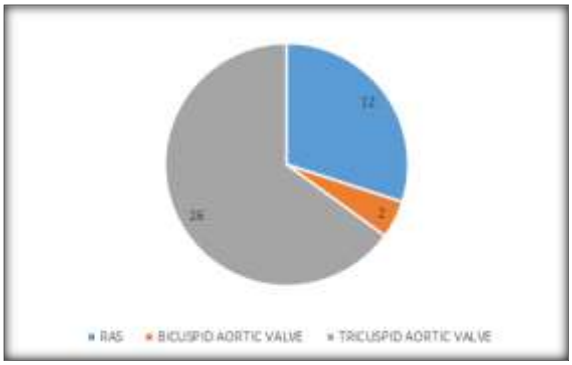

Figure 1: Type of valve involved in RAS and NRAS (N=40)

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.

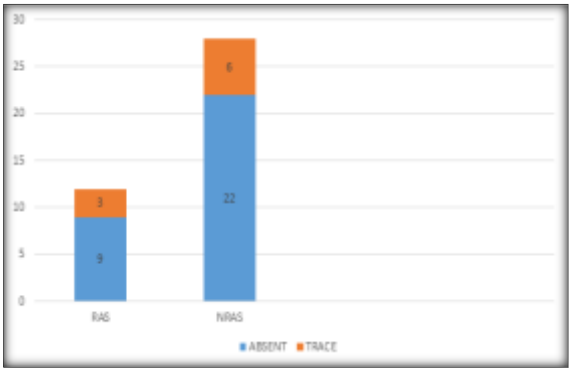

Figure 2: Grading for Haemorrhage(n=40)

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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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

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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.

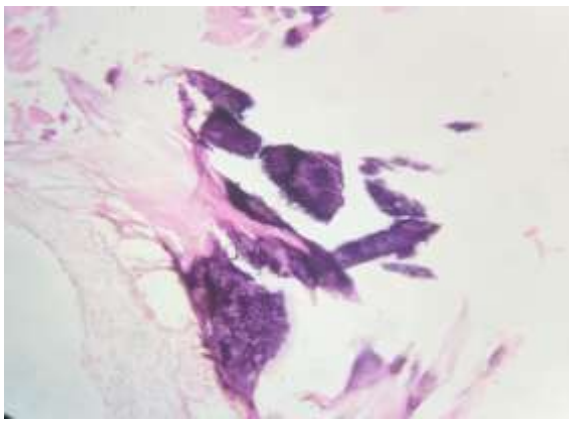

Figure 1: Microcalcification

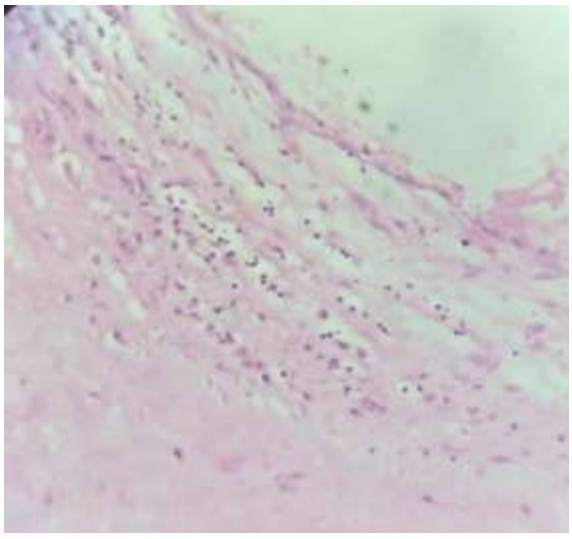

Figure 2: Mononuclear inflammatory infiltration

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.

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Original Research

Cytopathological Evaluation Of Image Guided Fine Needle Aspiration Cytology Of Liver Lesion With Cell Block Correlation.

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Abstract:

Background and rationale: Image guided fine needle aspiration cytology of liver lesion is a widely used procedure, providing rapid and accurate diagnosis. However, exact diagnosis is not possible always with information obtained by fine needle aspiration (FNA) cytological material and the possibility of indeterminate diagnosis exists. Therefore, we attempted to obtain additional information via the preparation of Cell block (CB) from the residual material of aspirates to aid in increasing the diagnostic accuracy.

Objectives:

1. To study cyto-morphological features in various hepatic lesions
2. To assess utility of cellblock preparation method in increasing sensitivity of cyto-diagnosis in liver lesion.

Study Design: Descriptive study

Methods and Material: We conducted a descriptive study at a tertiary care teaching hospital(Karnataka Institute of Medical Sciences, Hubli) for a period of one and half years which included a total of 75 cases with suspected liver mass. Using image guidance, fine needle aspiration was done and smears were prepared. The rest of the material present in the needle hub was submitted for cell block preparation.

Statistical analysis used: The quantitative variables were summarised using mean and qualitative variables as proportions. Sensitivity, specificity and positive predictive value were calculated for Image guided FNAC with Cell block as gold standard.

Results: Most common lesion was hepatocellular carcinoma. Sensitivity, specificity and diagnostic accuracy of smear preparation were 98%,90.2% and 96.6% respectively. With FNA and CB together, the sensitivity and specificity increased to 100%.

Conclusions: In this study, Image guided FNAC is found to be highly sensitive and accurate in diagnosing the hepatic lesions. Conventional smears can be adequate as a diagnostic tool in routine practices, but adding the cell block as an adjunct method will help us to increase the diagnostic accuracy and sensitivity especially in difficult to diagnose cases.

Key-words: Image guided FNAC, Liver lesions, Cell block

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Introduction:
The liver is one of the common organs for various non-neoplastic and neoplastic lesions. These include primary liver tumours, secondary deposits, cysts, abscesses and granulomas.
Image guided fine needle aspiration cytology (FNAC) is widely used, rapid, cost effective and minimally invasive method for the diagnosis of the liver lesions[1,2]. In addition to conventional smears, an attempt to obtain additional information can be made via the preparation of cell block from residual material remaining after completion of cytology preparation. This material often contains tissue fragments, which can give valuable information that cannot be processed by cytology[1,3]. Cytopathological evaluation and cell block preparation from FNA together yield two differing, complementary view of the same cell population[2].
The present study is undertaken to evaluate the cyto-morphological features of various hepatic lesions in detail as well as to assess the utility of cell blockpreparation method in increasing the diagnostic accuracy.

Methodology:

Primary objective: To study cyto-morphological features in various hepatic lesions.

Secondary objective: To assess utility of cellblock preparation method in increasing sensitivity of cyto-diagnosis in liver lesion.

Study Design: This is a descriptive study carried out in the cytology division of a tertiary care teaching hospital.

Study setting: Pathology Department of Karnataka Institute of Medical Sciences, Hubli, Karnataka.

Study duration: One and half years from October 2017 to March 2019 after obtaining the Ethical clearance from the Institutional Ethical Committee.

Study Subjects: Patients presenting with radiologically detected liver mass lesions were included in the study.

Exclusion Criteria: All abdominal mass cases other than liver lesion, patient with any bleeding disorder, non-co-operative patients and hydatid cyst diagnosed on ultrasound were excluded from this study.

Ethical considerations: Approval was obtained from institutional ethical committee prior to conducting the study(No.KIMS/PGS/SYN/447/2017-18).Informed written consent was obtained from each patient.

Method of data collection:
The patients were selected regardless of their age, sex, socio-economic status, and occupation. A detailed clinical history was taken and radiological findings were noted. Required tests like bleeding time, clotting time, prothrombin time and activated partial thromboplastin time were done. Under image guided techniques including Ultra-sonography (USG) and Computed tomography (CT), FNAC was done using lumbar puncture needle of 22 gauge. Minimum of 4 smears were prepared which included wet alcohol fixed smears for Haematoxylin & Eosin (H&E) and Papanicolaou's (PAP) stain, and air-dried smears for May-Grunwald Giemsa (MGG) stain. The rest of the material present in the needle hub and syringewere submitted in cell block solution (mixture of equal quantity of 100%

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ethanol and 10% formalin) using needle rinse method. Centrifugation of the sample was done at 2000 rpm for 2-3 minutes. Cell button submitted for routine histopathological examination. Paraffin embedded;4-6-micron thick sections will be routinely stained with H&E stain. Whenever necessary, histochemical special stains and Immunohistochemistry (IHC)were used.

Statistical analysis:
The data obtained was analysed by SPSS 21.0 software trial version. Microsoft word and excel was used to assimilate the data and prepare the article. The quantitative variables were summarised using mean and qualitative variables as proportions.

Results:
A total of 75 cases with liver lesion, clinically or radiologically diagnosed, were investigated. Our study showed a wide range of age distribution ranging from 22-85 years. The mean age of presentation was 58.9 years. Incidence of non-neoplastic lesion were predominantly seen in 5th and 6th decades and neoplastic lesions were more common in 6th and 7th decades. Out of the total 75 cases, 69.3% were males and 30.6% were females.
Majority of the patient presented with complaints of pain abdomen (74.67%). Most common imaging technique used was USG (95%) and in the remaining cases samples were obtained using CT guidance.
Out of 75 cases, adequate material was obtained on FNAC in 70 cases (93.3%). Adequatematerial for cell block was obtained in 60 cases (80%).
Out of the 70 cases with adequate material, 56 were neoplastic and 14 were non neoplastic cases.
Non neoplastic lesions included diffuse parenchymal liver disease, pyogenic abscess, regenerative nodule and simple cyst. Among these 56 neoplastic lesions, 35 were Hepatocellular carcinoma (HCC) and 20 cases were metastatic deposits and the remaining 1 case was diagnosed as haemangioma [Table 1].

Table 1.Type of lesions.

Lesions	Diagnosis	Frequency
Non neoplastic	Diffuse parenchymal liver disease	07(10%)
	pyogenic abscesses	04(5.7%)
	Regenerative nodule	01(1.42%)
	Simple cyst	02(2.85%)
Neoplastic	Hepatocellular carcinoma	35(50%)
	Metastatic deposits	20(28.5%)
	Haemangioma	01(1.42%)

NON NEOPLASTIC LESIONS
Most common non neoplastic lesion was diffuse parenchymal liver disease. The predominant pattern of arrangement of hepatocyte in these caseswere clusters and sheets. All the cases showed intra cytoplasmic bile pigment and presence of bile duct epithelium. Out of the 7 cases,5 cases showed fatty change. Cell block section showed sheets and groups of benign hepatocytes having abundant cytoplasm, central round nucleus with fine granular chromatin and prominent nucleoli. Micro vesicular and macro vesicular fatty changes were seen in the 5 cases.Special stain Masson trichrome stain was used in a case of diffuse parenchymal lesion presented with history of cirrhosis and it demonstrated blue stained collagen amidst the hepatocytes. Smears of pyogenic abscesses showed predominantly sheets of neutrophils and nuclear debris in a necrotic background. Clusters of benign hepatocytes were present. Cell block sections showed sheets of inflammatory cells and groups of hepatocytes.

PRIMARY NEOPLASTIC LESIONS

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Hepatocellular carcinoma was the most encountered lesion of the liver in the present study (50%).Detailed cyto-morphological analysis of each case was done and compared with cell block findings[Table 2].
Among 35 cases, aspiration was highly cellular in 26 cases and was moderate in remaining 9 cases. Out of the 35 cases, 20 cases showed classic trabecular pattern. It was the commonest pattern seen in well differentiated HCC. Other patterns commonly encountered were clusters and dispersed single cell arrangement. Clusters of hepatocytes with traversing blood vessels were seen in 30 cases (85.7%). Endothelial wrapping around the trabecula were seen in 15 cases (42.8%). Pleomorphic nuclei were observed in 88.5% of the cases. All the cases showed high nuclear cytoplasmic ratio, prominent macro nucleoli and atypical naked nuclei(100%). Intra nuclear cytoplasmic inclusion was present in 23 cases (65.7%). Presence of intracytoplasmic bile pigment could be appreciated in 8 cases (22.8%) and bile duct epithelium was absent in all the 35 cases.

Table 2. Cytomorphological analysis

Cytological feature	Number of cases
High cellularity	26 (74.3%)
Trabecular pattern	20 (57%)
Traversing blood vessels	30 (85.7%)
Endothelial wrapping	15 (42.8%)
High N/C ratio	35 (100%)
Pleomorphism	31 (88.5%)
Coarse chromatin	35 (100%)
Prominent nucleoli	35 (100%)
Naked nuclei	35 (100%)
Intra nuclear inclusion	23 (65.7%)
Intra cytoplasmic bile	08 (22.8%)
Absent bile duct epithelium	35 (100%)

Hepatocellular carcinoma cases were further sub classified depending on these cytological and nuclear features. Out of these 35 cases, 8 were well differentiated HCC [Figure 1], 24 were moderately differentiated HCC and 3 cases were poorly differentiated HCC.

The well differentiated HCC showed high cellularity in majority of the cases with tumour cells arranged predominantly in trabecular pattern (75% of well differentiated HCC). These tumour cells were showing absent or mild pleomorphism with abundant granular cytoplasm, central round nuclei with coarse chromatin, prominent nucleoli and slightly increased nuclear cytoplasmic ratio. Endothelial wrapping was present in the 7 cases of well differentiated HCC (88%). Traversing blood vessels was present in 6 cases of well differentiated HCC (75%). The predominant pattern identified in cell block sections were trabecular pattern. Thickness of trabeculae was more than 3-4 cell thickness in all the cases.
The moderately differentiated HCC showed cellular smears with tumour cells arranged predominantly in clusters and in trabecular pattern. These tumour cells were moderately pleomorphic with high N/C ratio, round to oval nucleus with coarse granular chromatin, multiple macro nucleoli and moderate cytoplasm. Traversing blood vessels was present in 22 cases of moderately differentiated HCC (92%). Endothelial wrapping was present in the 8 cases of moderately differentiated HCC (33.3%). Cell block showed tumour cells arranged in small groups and trabecular pattern.
The cases of poorly differentiated HCC showed discohesive tumour cells arranged in dispersed singles. These tumour cells were having scant cytoplasm, marked nuclear pleomorphism and multiple macro nucleoli. Multi nucleated tumour giant cells were frequently observed. Cellblock sections showed dispersed tumour cells in singles and small groups.

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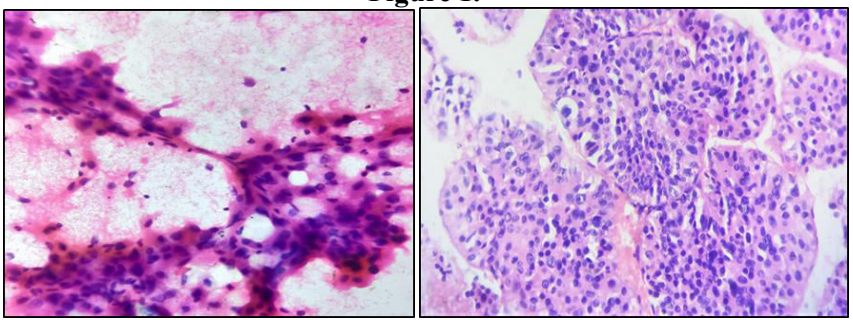
Out of 35 cases, 20 cases were confirmed with IHC using marker HepPar 1. These cases showed diffuse granular cytoplasmic positivity.

Figure 1.

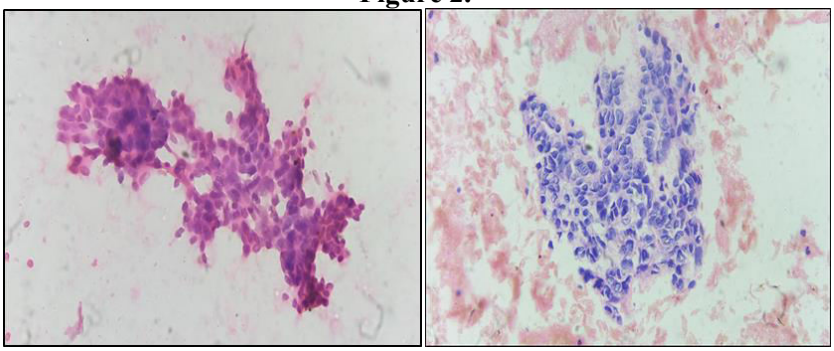


Well differentiated HCC (FNAC- H&E,40X,CB-H&E,20X)

METASTATICDEPOSITS

The remaining 20 cases out of the 56 neoplastic lesionswere reported as metastatic deposits to the liver. Of these, 18 cases were metastatic adenocarcinoma deposits [Figure 2], 1 case was metastaticadeno-squamous carcinoma deposit and the remaining 1 case was malignant melanoma deposit. Most common primary site of malignancy in this study was Colon (6 cases) followed by pancreas (4 cases), oesophagus (3 cases), breast (2 cases), ovary (1 case) and lung (1 case).

Figure 2.



Metastatic deposits from Papillary Carcinoma Ovary (FNAC-H&E,40x & CB-H&E,40x)

Most common pattern of arrangement of tumour cells observed in these metastatic deposits were clusters followed by acinar pattern and papillary pattern.

The present study predominantly comprised of metastatic adenocarcinoma deposits (90% of metastatic deposits). In adenocarcinoma deposits, tumour cells were cuboidal to columnar with hyperchromatic nuclei and moderate amount of vacuolated cytoplasm. Deposits from colon carcinoma showed intracellular and extra cellular mucin in the background. Cell block sections showed tumour cells arranged in clusters, acini and papillary pattern and having hyperchromatic nuclei and vacuolated cytoplasm. Special stain with PAS showed intracytoplasmic positivity.

Deposit from malignant melanoma showed large tumour cells with irregular nucleus, prominent nucleoli and moderate cytoplasm containing melanin pigments. Cell block sections showed sheets of tumour cells with intracellular and extra cellular melanin pigments. These cells showed strong cytoplasmic positivity with HMB-45 in immunohistochemistry [Fig 3]

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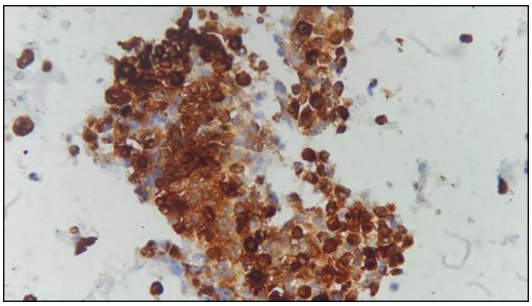
FIGURE 3



IHC on cell block section showing intracytoplasmic positivity for HMB-45 in malignant melanoma

Out of the 20 cases, 3 cases were of unknown primary. Clinical details of these cases werecollected and an attempt was made to predict the primary site of tumour by assessing the cyto morphology in smears, histo-morphology and IHC in cell block [Table 3]

Table 3.Cytomorphology in smears, histomorphology and IHC in cell block a comparison

CLINICAL DIAGNOSIS	FNAC DIAGNOSIS	CB DIAGNOSIS	IHC
CT; pancreatic solid lesion	Metastatic adenocarcinoma deposits	Adeno squamous deposits	CK5 +, CK7+
Suspected case of primary Carcinoma stomach	Metastatic adenocarcinoma deposits	Metastatic adenocarcinoma deposits	CK7+
Metastatic adenocarcinoma deposits	Metastatic adenocarcinoma deposits	Cholangiocarcinoma	Inadequate

ANALYSIS OF EFFICIENCY OF SMEARS

Diagnostic sensitivity, specificity and positive predictive value of FNAC smears were calculated considering cell block as gold standard[Table 4].

Table 4. Diagnostic test evaluation parameters of FNAC

Diagnostic test parameter	Value
Sensitivity	98%
Specificity	90.2%
Positive predictive value	96.6%

Table 5.Comparison of cytological features of HCC in different studies

Cytological features	Shashikala V et al[12]	Cohen et al[19]	**Present study**
High cellularity	-	83%	**74.3%**
Trabecular pattern	50%	65%	**57%**
High N/C ratio	-	71%	**100%**

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Naked nuclei	65%	73%	**100%**
Prominent nucleoli	100%	54%	**100%**
Intra nuclear inclusion	45%	-	**65.7%**
Intra cytoplasmic bile	15%	-	**22.8%**
Coarse chromatin	-	33%	**100%**
Pleomorphism	-	71%	**88.5%**
Absence of bile duct epithelium			**100%**

In 7 cases (10.3%) with difficult or doubtful diagnosis in smears, CB helped to arrive a definitive diagnosis. Difficulties faced in the present study was differentiating non neoplastic lesions from well differentiated HCC, differentiating poorly differentiated HCC from metastatic deposits and detection of the origin of metastatic deposits.Whichever cases had difficulties in differentiating the well differentiated HCC and non-neoplastic liver diseases using smears, we used cell block to confirm the diagnosis. Solid pattern and trabeculae of hepatocytes thicker than 4 cells rimmed by endothelial cells were considered as a feature of HCC.Differentiating poorly differentiated HCC from poorly differentiated metastatic tumour is challenging. IHC using Hep Par-1 antibody was performed in doubtful cases to confirm the diagnosis.
With FNA and CB together, we were able to provide a definitive cyto-pathological diagnosis in all the 60 cases, increasing the sensitivity to 100%.

Discussion:
Fine needle aspiration cytology of the liver was established by Sodenstorm in 1966 with the examination of 500 cases[4].Later in 1976, Haaga et al introduced a method of preciselocalization of lesion by Computed tomography (CT). This allowed accurate positioning of needle in small and deep lesions[5].Over the last 15 to 20 years, FNAC under image guidance has attained an increasing acceptance as the diagnostic procedure of choice for single or multiple focal hepatic lesion.
The use of cell block for processing cytology fluids has been first reported in 1895 by Bahrenberg[6,7]. In addition to the conventional smears, cell block technique has a significant role in diagnostic cytopathology and it is a valuable adjunct towards a more accurate cytological diagnosis complementary to smears and histology[6,7,8]. Cell block preparation are used routinely for body fluids, fine needle aspirations and other cytological samples.
Computed tomography(CT) and ultrasound guidance (USG) are the two main image guidance system using for liver FNAC. In this present study, ultrasonography was the main guidance technique we have used.
In the present study, the mean age at presentation was 58.9 years ranging from 22-85 years which is comparable with the study done by Haqsheefa et al[10] in which the mean age of presentation was 58.8 years. Maximum number of cases were seen between 51 and 70 years of age (6th and 7th decade) (70%) and this is comparable with the study done by Mathew and Nair[11] in 2017 and Shashikala V et al.[12] in 2016. Our study showed a male predominance with male to female ratio of 2.3:1 and these results are similar to the studies done by Haqsheefa et al[10] and Shashikala V et al[12].
Most important requirement for cyto-diagnosis is to obtain a representative sample[13]. In the present study, satisfactory aspirate for cyto-diagnosis on FNAC was obtained in 70 cases (93.3%). This is comparable to 91.67% adequacy obtained in a study done by Rajesh Chandan et al[14]and 96.96% adequacy obtained in the study done by Shashikala Vinayakamurthy et al[12].Adequate material for cell block was obtained in 60 cases (80%) and this is comparable to the study by Nathan et al.[15] who obtained adequate material on cell block in 73.3% cases. Major factor contributing to the optimal preparation of cell block is adequate rinses from fine needle aspiration syringe to extract the residual tissues.
Out of the 70 cases studied, majority of the lesions were neoplastic (80%) and the remaining 20% were non neoplastic lesions. This is similar to the distribution of cases found in the study by Balani et al.[13](2013), study by Rajesh Chandan et al.[14](2018) and study by Shashikala Vinayakamurthy et al.[12].

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Most common non-neoplastic lesion identified in the current study was diffuse parenchymal liver diseases (10%). This is similar to the study conducted by Rajesh Chandan et al.[14]and the study conducted by Asghar F and Riaz S[16].In an another study conducted in Bangalore, Karnataka by Shashikala V et al[12], most common non-neoplastic lesion identified was pyogenic abscess.

The present study showed 50% of Hepatocellular carcinoma, 28.5% of metastatic carcinoma and 1.4% of haemangioma. Hence, the most common lesion of the liver in our study was hepatocellular carcinoma (HCC). This is comparable with different studies done by Shashikala Vinyakamurthy et al[12]., Mohammed AA et al.[1] and Sumana BS[3] in which the majority of cases were HCC. However, our study differs from the other studies done by Khanna et al.[17], Rajesh Chandan et al.[14] and Nosher et al.[18] since the majority of cases in their studies were metastatic deposits.

HCC is the most common primary malignancy of the liver and early diagnosis of HCC is important because of prognostic implication[10].Cohen et al.[19], proposed three primary useful criteria to discriminate between HCC and non- neoplastic liver lesion in FNAC. These features were increased nuclear cytoplasmic ratio (N/C), trabecular pattern and atypical naked nuclei.In the current study, we examined 10 cytological features that have been reported as useful in the literature for diagnosis of HCC. We analysed the utility of these features in all the 70 cases of liver FNAC. The current study identified the following features in all the cases of HCC; atypical naked nuclei in the background, presence of macro nucleoli, absence of bile duct epithelium, high N/C ratio and coarse clumped chromatin. These were the main features used to differentiate between HCC and non-neoplastic liver diseases in the present study. In addition to that, features like high cellularity and trabecular pattern are also considered. Useful features we found in the present study to differentiate between HCC and metastatic deposits are trabecular pattern, atypical naked nuclei, presence of macro nucleoli, intra nuclear inclusion and intra cytoplasmic bile pigment.

Based on above mentioned cytological features, all the cases of HCC were further sub-classified into 3 grades; Well differentiated HCC (8 cases), moderately differentiated HCC (24 cases) and poorly differentiated HCC (3 cases). This is comparable with the study conducted by Haq Sheefa[10] where majority of the HCC cases were moderately differentiated.However, one study conducted in Bangalore, Karnataka by Shashikala V et al[12] showed predominance of well differentiated HCC. Another study conducted in Bhopal by Balani et al[13] showed predominance of poorly differentiated HCC.

In the present study, metastatic deposits to the liver were accounting for 28.5% and major bulk of metastatic tumours in the present study comprised of metastatic adenocarcinoma deposits in 90% of metastatic deposits.This is comparable with the study done by Shashikala V et al.[12]and another study done in Bhopal, Madhya Pradesh by Balani et al[13], 89.6% of the metastatic deposits were adenocarcinoma deposits.The most common primary site of malignancy identified in the present study was colon and pancreas. This is comparable with the studies done by K.Ceyhan et al.[2] and Balani et al.[13]

The distinction between a primary carcinoma liver and metastatic deposits is very important since it has both therapeutic and prognostic significance.The cytological criteria to differentiate HCC from metastatic tumours stated by Bottles et al.[20] include polygonal cells with centrally placed nuclei, malignant cells separated by sinusoidal capillaries, presence of bile, intra-nuclear cytoplasmic inclusions and endothelial rimming.The salient features separating HCC from metastatic adenocarcinoma deposits described by Greene et al[22] were tumour cells in HCC are polygonal or polyhedral, have abundant eosinophilic and granular cytoplasm, macro nucleoli and trabecular arrangement.Whereas tumour cells in metastatic adenocarcinoma deposits are columnar or cuboidal, predominantly have acinar or glandular arrangement, show mucin secretions and inflammatory background.The salient features separating HCC from metastatic adenocarcinoma deposits identified in the current study were also the same.

Deposit from Metastatic melanoma may mimic HCC since tumour cells have several similar features. Also, melanin pigment can resemble various liver cell pigments. History of primary lesion elsewhere and features of melanoma like single cells with eccentric nucleus, single prominent nucleoli, bi-

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nucleation may help distinguish it from HCC. Immunohistochemistry can be done to confirm the diagnosis.

Various studies have reported sensitivity varying from 62-100% and specificity 63% to 100%[1,2,12,13]. In the present study FNAC was able to diagnose the liver lesion with an overall sensitivity of 98% and specificity of 90.2%. The overall accuracy of procedure in the present study was 96.6% which was comparable to the rate of accuracy reported in the studies done by Mohmmed AA et al[1] and Shashikala V et al[12].

The utility of cell block along with routine smears has been evaluated by various authors. In the present study, cell block provided additional information in 10.3% of the cases. In a study done by Liu et al[21], cell block section provided additional information in 12% of the cases andanother study by Nathan et al.[15], described 15.2% of improvement in diagnosis when both smears and cellblocks were studied together.In another study conducted in Bangalore, Karnataka by BS Sumana and Bharathi Muniyappa[3], cell block helped to improve the diagnosis in 15.55% of the cases.

With smear and cell block together, 100% correct diagnosis was achieved in all the 60 cases increasing the sensitivity and specificity to 100%. This is comparable with the study done by Shashikala V et al.[12] The result of this study revealed that diagnostic sensitivity can be increased by adding cellblock as adjunct method along with conventional smears.

Cell block sections displayed cytomorphology clearly recognizable with limited shrinkage. The cyto-morphological features were properly maintained with clear recognition of nuclear and cytoplasmic features. This is corresponding to study done by Kung et al.[23] regarding the staining results on CB where cell block sections showed excellent staining results with IHC.In addition to the role in increasing diagnostic sensitivity, cell block section can also be used for special stains and immunohistochemistry. In the current study, cell block sections showed excellent staining pattern with IHC markers.

Conclusions:

In this study, Image guided FNAC is found to be highly sensitive and accurate in diagnosing the hepatic lesions. Conventional smears can be adequate as a diagnostic tool in routine practice, but adding the cell block as an adjunct method will help us to increase the diagnostic accuracy and sensitivity especially in difficult cases.

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