

Original Article

Histopathological evaluation of chronic rheumatic mitral valve stenosis: the association with clinical presentation, pathogenesis, and management at a National Cardiac Institute, Tanzania.☆☆



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ABSTRACT

Aims: The histopathology of mitral valve (MV) tissues have been reported in necropsy and retrospective studies. We prospectively studied the histopathological changes in rheumatic mitral stenosis using advanced techniques and corroborated these with clinical presentation, pathogenesis, and management. **Methods:** From January 2020 to February 2021, surgically excised rheumatic stenotic MV from 54 Tanzanian patients were studied. These were examined using hematoxylin-eosin, von Kossa staining, and immunohistochemistry.

Results: The median (range) age of patients was 39 (14–57) years with 34 (63%) females. Secondary prophylaxis was given to 7 (13%) patients and 2 (3.7%) had evidence of rheumatic fever (RF). With hematoxylin-eosin, 37 (68.5%) specimens showed fibrinoid degeneration (FD), 44 (81.5%) leucocytic infiltrates, 6 (11.1%) Aschoff nodules, 30 (55.6%) calcification, and 39 (72.2%) fibrosis. Thirty-five (64.8%) specimens were positive to von Kossa. The proportion of specimens positive for CD3, CD20, CD68, and CD8 were 46 (85.2%), 35 (64.8%), 39 (72.2%), and 8 (14.8%) respectively. Valvular calcium was high among older patients, males and with a higher trans-MV gradient. The degree of inflammatory cellular infiltration was associated with valvular calcification, FD with ARF, leucocytic infiltrates with disease duration of <10 years, and fibrosis with the absence of atrial fibrillation. C-reactive protein and anti-streptolysin titres were high in CD20 and CD8 staining cells.

Conclusion: This study confirms that high MV calcium are found in patients who are old, male, and with severe mitral stenosis. The association between clinical parameters with histopathological-immunohistochemical studies observed in our study provides new insight to disease presentation. We found a low rate of secondary prophylaxis and two patients with ARF. Our findings are comparable with those from other countries suggesting similar pathogenesis and thus intervention modalities. This is the first study on mitral valve histopathology to be reported from Africa.

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1. Introduction

Rheumatic heart disease (RHD), an important cause of cardiac morbidity and mortality among children and young adults affecting an estimated 15.6 million people yearly and annually responsible for 300,000 deaths worldwide [1], has a sub-Saharan Africa prevalence ranging from 5.7 to 21.0 per 1,000 school children [2–5].

Surgically excised mitral valve (MV) tissue provides information related to the severity of disease, clinical presentation, and insight into pathogenesis and management [6–9]. For example, higher amounts of calcium are commonly found in men, in older patients and patients with higher trans-MV pressure gradients [9–11]. In addition, there is a higher correlation between the degree of valvular calcification and the extent of inflammatory response [7,8,12]. Neo-angiogenesis, calcific deposits, and inflammation play a role in the formation of valvular calcification [7,12]. The Aschoff granuloma, when present, signify acute rheumatic fever (ARF) but do not necessarily indicate an active attack [9,13–17]. On the other hand, presence of fibrinoid degeneration (FD) implies an acute phase while the histiocytes and giant cells indicate the chronic phase of the disease [9,16].

Heart valves from RHD patients reveal marked inflammatory cellular infiltrates. These cells are capable of producing cytokines and soluble molecules that affect valvular interstitial and endothelial cell activities [14,18,19]. Macrophages are plenty and play a key role in the production of inflammatory mediators that are implicated in the remodelling of extracellular matrix and fibrosis [6,20]. Similarly, B- and T-lymphocytes have been found in abundance in the heart valves of RHD patients. Furthermore, in ARF/active RHD, proinflammatory cytokines have been reported [18] and are associated with increased numbers of CD4⁺, CD8⁺ and CD25⁺ cells [6,21,22].

The histopathological findings in the valvular tissues from RHD patients have been previously reported.[7,14–17,21,23–25] However, these studies are: descriptive necropsy,[15,24,26,27] retrospective,[15,16,23,28] used conventional histology,[7,15,16,23,24] were done outside Africa[7,14,15,17,21,24,28] and many decades ago.[7,15,17,21,24] We aimed to conduct a prospective study of heart valves in RHD patients focusing on the histopathological changes with clinical presentation, disease pathogenesis, and management.

2. Material and methods

2.1. Study design and setting

This was a prospective study on surgically excised rheumatic mitral stenosis (rMS) valves from Tanzanian patients. All consecutive patients who were accepted for mitral valve replacement (MVR) due to moderate-severe rMS (from January 2020 to February 2021) were enrolled. We excluded patients with other forms of valvular heart disease or other cardiac diseases. The specimens were collected from patients admitted and operated on at the Jakaya Kikwete Cardiac Institute (JKCI) in Dar es Salaam, Tanzania. The specimens were submitted to the histopathology unit at Muhimbili National Hospital. Clinical and surgical information of each specimen was provided to two pathologists who performed all histopathological evaluations independently and/or by consensus.

2.2. Clinical evaluation

The past medical and comorbidity history was obtained from all patients. Patients were clinically evaluated for the evidence of moderate to severe MS according to recognized clinical and

echocardiographic criteria [29]. Preoperatively, Doppler echocardiography was used to make a diagnosis. The diagnosis of ARF was made based on the recognized criteria [30]. The diagnosis of atrial fibrillation was made on a clinical basis and confirmed by electrocardiogram.

2.3. Histopathological studies

2.3.1. Biopsies

The excised MV tissues were grossly evaluated and fixed in 10% well-buffered neutral formalin and then paraffin-embedded. Subsequently, a critical gross examination of the valves was performed at the histopathology unit by a cardiologist and a pathologist following established macroscopic criteria for rheumatic carditis [23,31]. and representative MV specimens were photographed. Tissue processing and eventually decalcification, sectioning and staining were done by specialist histotechnologists using the Sakura Tissue processor and sectioning (4 μ m) by using Sakura Rotary Microtome 200.

2.3.2. Routine staining

Hematoxylin-eosin (H&E) staining was done on formalin-fixed and paraffin-embedded (FFPE) biopsies after tissue processing and microtomy as previously described [32]. Briefly, FFPE sections were dewaxed in two changes of xylene for five minutes in each and rehydrated to water through descending grades of ethanol (100%, 95%, 80%, and 70%) ten dips in each followed by staining in Harris' hematoxylin for ten minutes. Thereafter, slides were briefly rinsed in tap water and differentiated in 1% acid-alcohol for 30 seconds. Bluing of slides was done in running tap water for ten minutes and then counterstained in 1% aqueous eosin for 4 minutes. Slides were then washed in tap water, dehydrated in ascending grades of ethanol (70%, 80%, 95%, and 100%) ten dips in each and cleared in two changes of xylene for 10 minutes and mounted by using an automated Sakura TCA 200 cover-slipping and labelled accordingly.

2.3.3. Histopathological microscopic evaluation

The H&E-stained sections of MV were evaluated for the presence of fibrinoid degeneration in leaflets or annular tissue, leucocytic inflammatory cell infiltrates, oedema, neovascularization, Aschoff nodules, calcification, and fibrosis. These were either noted for presence or absence.

2.3.4. Histochemistry

The von Kossa histochemical staining was done according to the protocol previously described [32]. Briefly, the 4 μ m thick tissue sections were allowed to float in a distilled water bath at 45°C and mounted on frosted glass slides, dried and then incubated in a hot air oven for 30 minutes at 60°C followed by dewaxing in two changes of xylene for 3 minutes in each and rehydrated to distilled water. Slides were then placed in 10% silver nitrate solution and exposed directly underneath a 100W electric light bulb for one hour. Thereafter, slides were rinsed in two changes of distilled water followed by immersing in 5% sodium thiosulphate solution for 5 minutes and then counterstained in 1% neutral red for 1 minute, blot dried, dehydrated in ascending grades of alcohol, cleared in xylene and mounted.

2.3.5. Histochemical microscopic evaluation

Trephine biopsy and tissue without calcium were used respectively as positive and negative controls and were included in each batch of staining. A semi-quantitative grading system was used as described by Subramanian et al [33] and Lars et al [14]. The extent of valvular calcification was graded as: absent, mild, moderate, severe and the degree of microcalcification as 0 = absent,

trace/mild = scattered/dense deposits covering ≤ 2 HPF, moderate = dense deposits in 3–6 HPFs, or severe = dense deposits in ≥ 6 HPFs.

2.3.6. Immunohistochemistry (IHC)

Immunohistopathological studies for B-lymphocyte (CD20), total T-lymphocyte (CD3), macrophage (CD68), T-helper lymphocyte (CD4), cytotoxic T lymphocyte (CD8), and regulatory T lymphocyte (CD25) markers were performed as previously described [32]. Briefly, the 3 μ m thick tissue sections were labelled with respective antibodies and incubated in a hot air oven overnight at 40°C. The next day the slides were deparaffinised using two changes of xylene for 8 minutes, then rehydrated using decreasing grades of ethanol. Sections were first blocked with peroxidase blocking reagent (Dako Carpinteria, CA) for 15 minutes followed by antigen retrieval with citrate buffer pH 6.0 in a pressure cooker for 20 minutes at 100°C. The plastic container with retrieval solution and slides was placed into the pressure cooker with the lid being tightened then heating the tap water for 20 minutes. Sections were then stained with antibodies for CD3 (clone polyclonal, RTU, Dako), CD4 (clone 4B12, RTU, Dako), CD8 (clone C8/144B, RTU, Dako), CD20 (clone L26, RTU, Dako), CD25 (clone EP 218, RTU, USA) and CD68 (clone PG-M1, Dako; 1:10) for 30, 25, 32, 28, 32, and 35 minutes, respectively. Thereafter, sections were incubated with secondary antibody for 25 minutes (horse-raddish peroxidase [HRP] detection system, Dako Carpinteria, CA) and visualized by 3, 3'-Diamino Benzidine (DAB by Dako). Slides were then washed in three changes of wash buffer for 9 minutes to make sure HRP is completely removed. Sections were then incubated with DAB for 10 minutes followed by rinsing in water for 2 minutes then counterstained with hematoxylin for 1 minute, briefly differentiated in 1% acid alcohol and blued for five minutes. Sections were dehydrated in the ascending grades of alcohol and then cleared in two changes of xylene for 5 minutes in each and covered by using a cover slip-per.

2.3.7. Microscopic IHC evaluation

Normal tonsil tissue was used as a positive control for the antibodies, and the negative control; the primary antibody incubation step was replaced by a phosphate buffer solution as previously described [32]. Nonstaining parts/cells within the same tissue were used as internal negative controls as previously described [32]. A semi-quantitative grading system was applied for the degree of mononuclear inflammatory cell infiltration as previously described [34] as 0, 1+, 2+, or 3+.

2.4. Statistical Analyses and ethical consideration

Data analysis were done by using Prism V.8.0.1 and SPSS V.27. Continuous data was presented as median or mean and discrete data as counts. The Chi-square and Fisher's exact tests were used to compare the histopathological findings of the excised MV with the clinical findings and results for complex data were presented by the heat map charts. Kappa statistics were used to test the reliability and agreement between the two tests. A *P*-value of ≤ 0.05 was considered statistically significant. We obtained written informed consent from all participants. The study was approved by the institutional review board through the Directorate of Research and Publications of Muhimbili University of Health and Allied Sciences (P. MUHAS - REC-9-2019-059).

3. Results

Fifty-four Tanzanian patients, who were consecutively accepted for MVR at JKCI due to moderate to severe rMS were enrolled in the study. There was a female 34(63%) predominance among the

Table 1

Baseline characteristics of patients operated for Rheumatic MS at JKCI from January 2020 to February 2021 (*N* = 54).

Variable	Mean (\pm SD)/frequency (%)
Age (years)	37.9 \pm 12
Female sex	34 (63)
Mean duration of symptoms (years)	6.83 \pm 5.51
Duration of symptoms categories (years)	
<10	36 (66.7)
≥ 10	18 (33.3)
Diagnosis	
Pure MS	19 (35.2)
Mixed mitral valve disease	35 (64.8)
Proportion with RF	2 (3.7)
Proportion with atrial fibrillation	24 (44.4)
Proportion with NYHA class III-IV	15 (27.8)
Proportion received Benzylpenicillin	7 (13.0)
Proportion with stroke	9 (16.7)
Proportion with hypertension	6 (11.1)
Proportion with acute rheumatic fever	2 (3.7)
Proportion with anaemia	1 (1.9)
Proportion with diabetes	1 (1.9)
Proportion with HIV	1 (1.9)
Mean Wilkins' score	11.76 \pm 1.74
Mean mitral valve area (cm ²)	1.14 \pm 0.39
Mean trans-mitral pressure gradient (mmHg)	12.54 \pm 3.57
Mean left atrium volume index (ml/m ²)	90.46 \pm 30.62

HIV = human immunodeficiency virus; MS = mitral stenosis; NYHA = New Heart Association; RF = rheumatic fever.

recruited patients for the study. Their median (range) age was 39 (14– 57). Twenty-nine (53.7%) patients had severe MS, 18 (33.3%) had disease duration of >10 years, 35 (64.8%) mixed (with mild mitral regurgitation) mitral valve disease, 2 (3.7%) ARF, 7 (3.7%) were on secondary prophylaxis and 24 (44.4%) had atrial fibrillation. Their mean Wilkins' score was 11.76 \pm 1.74 (Table 1). Seventeen (31.5%) patients had elevated anti-streptolysin titres (ASOT), 5 (9.3%) had prolonged PR interval on electrocardiogram, and 33 (61.1%) had elevated C-reactive protein (CRP).

The proportion of specimens that stained on H&E showed Aschoff nodules (Fig. 1A–C) and Anitschkow cells (Fig. 1B and C) in 6 (11.1%) specimens, 44 (81.5%) showed leucocytic inflammatory cell infiltrate (Fig. 1D and E), 18 (33.3%) neovascularization (Fig. 1D and E), 37 (68.5%) edema (Fig. 1D,E&F), 28 (51.9%) haemorrhage (Fig. 1F), 30 (55.6%) calcification (Fig. 1G and H), 37 (68.5%) fibrinoid degeneration (Fig. 1I), and 39 (72.2%) fibrosis. Furthermore, in our current cohort, the histopathological changes observed did not seem to show a specific pattern of distribution relative to the valvular microarchitecture neither did they seem to be related to the flow vs. non-flow surfaces of the valves.

With von Kossa stain, 35 (64.8%) of the tissues showed evidence of calcification (Fig. 2A) of which 18 (33.3%) were moderate to severe. The proportion of tissues stained with immunohistochemical markers were: 39 (72.2%) with CD68, 46 (85.2%) with CD3, 35 (64.8%) with CD20 (Figs. 2B–D respectively), and 8 (14.8%) tissues with CD8. None of the tissues were stained with markers for CD4 and CD25.

Table 2 shows statistically nosignificant higher MV calcium levels among patients with age of >30 years, males, and with a higher trans-MV pressure gradient. Patients with a duration of symptoms <10 years had higher MV calcium than those with duration ≥ 10 years. When present, valvular calcification was more likely to be detected by echocardiography than not to be detected. The percentage in agreement for detecting valvular calcification between echocardiography and von Kossa stain was 66.6% (kappa value 0.269, *P*=.048). The mean Wilkins' score among patients without valvular calcium was higher than those with calcium.

Table 3 depicts a statistically significant association (and a trend towards the increase) between the extent of calcification and the

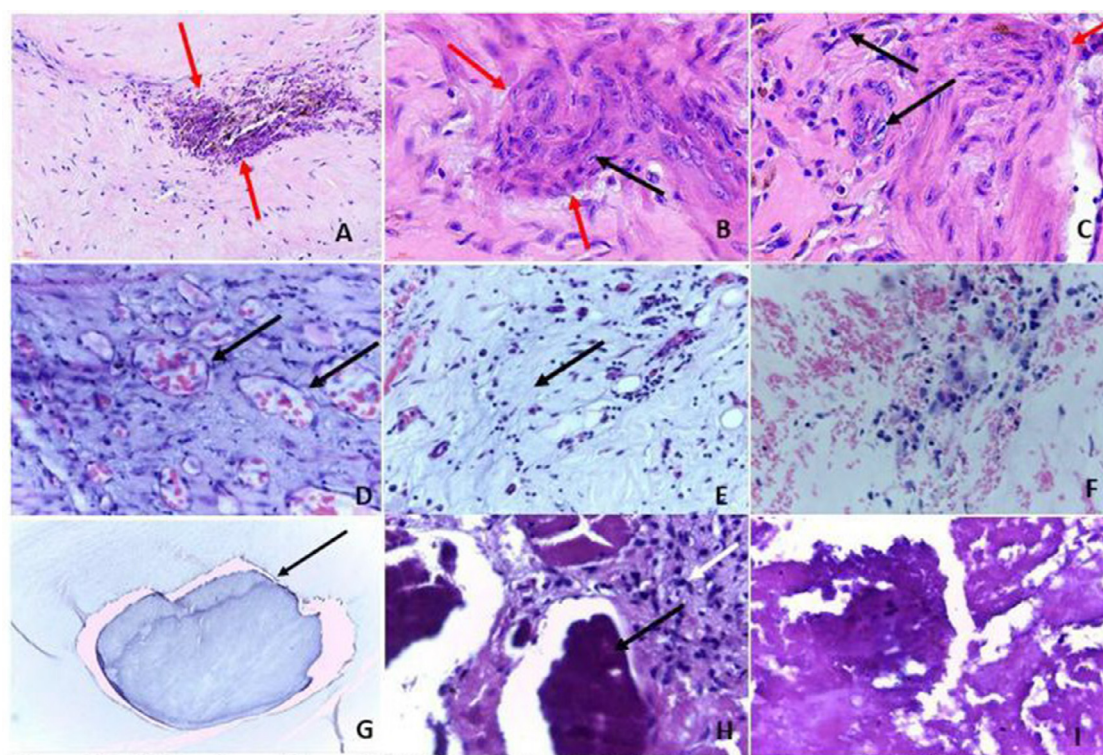


Fig. 1. H&E-stained microphotographs of sections from patients with rheumatic MV stenosis showing: Aschoff bodies (A-C) red arrows and Anitschkow cells with caterpillar-like chromatin (B and C) black arrow; valvulitis (D and E) with neo-angiogenesis, vasocongestion and granulation tissue (D and E). Valvular oedema (D-F); haemorrhage (F); macrocalcification (G and H) black arrows and valvulitis leading to calcification (H) white arrow; extensive fibrinoid degeneration (I). H&E = hematoxylin-eosin. (Color version of figure is available online.)

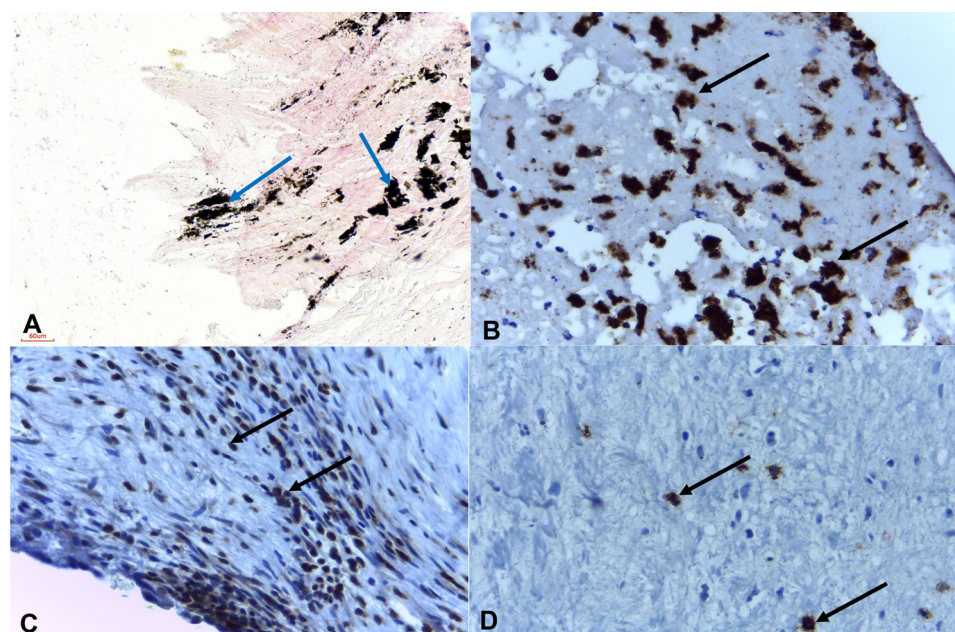


Fig. 2. von Kossa stained MV tissue specimen (A) showing intense calcification and photomicrographs of excised MV tissues stained by immunohistochemistry with anti-CD68 (B), anti-CD3 (C), and anti-CD20 (D) monoclonal antibodies.

degree of cellular infiltrations as marked by CD3, CD20, and CD68 staining. For CD8 marker the association was statistically not significant.

Table 4 shows a statistically significant association (and a trend towards severity) between calcification and the degree of cellular infiltrations as marked by CD3, CD20, and CD68 staining. For CD8 marker, the association was not statistically significant.

Fig. 3 shows a statistically significant difference in presence of: fibrinoid degeneration in patients with ARF vs without (91.3% vs 51.6%, $P=.003$); leucocyte infiltrates among patients with disease duration <10 years vs ≥ 10 years (91.7% vs 61.1%, $P=.011$); and fibrosis in patients without vs with atrial fibrillation (86.7% vs 54.2%, $P=.014$). The remaining histopathological findings did not reveal a statistically significant association with the analysed parameters.

Table 2

Association between presence of valvular calcium with clinical variables among the operated patients (N = 54).

Variable	Category	Presence of valvular calcium		P-value
		Yes n (%)	No n (%)	
Age (years)	≤30	10 (62.5)	6 (37.5)	.817
	>30	25 (65.8)	13 (34.2)	
Sex	Male	16 (80.0)	4 (20.0)	.073
	Female	19 (55.9)	15 (44.1)	
Mean PG	Moderate	5 (50.0)	5 (50.0)	.277
	Severe	30 (68.2)	14 (31.8)	
Duration of disease (years)	<10	27 (75.0)	9 (25.0)	.027
	≥10	8 (44.4)	10 (55.6)	
Atrial fibrillation	Yes	15 (62.5)	9 (37.5)	.750
	No	20 (66.7)	10 (33.3)	
ECHO calcification	Yes	26 (74.3)	9 (25.7)	.048
	No	9 (47.4)	10 (52.6)	
Mean Wilkins' score		11.40 ± 1.61	12.42 ± 1.81	0.038
Mean MVA		1.07 ± 0.35	1.26 ± 0.43	0.082

ECHO = echocardiography; MVA = mitral valve area; PG = pressure gradient.

Table 3

Association between the extent valvular calcification and the degree of inflammatory cell infiltrations among the operated patients (N = 54).

Variables		Extent of valvular calcification				P-value
		Absent (%)	Mild (%)	Moderate (%)	Severe (%)	
CD3	Absent	6 (31.6)	2 (8.3)	0 (0.0)	0 (0.0)	.002
	Occasional	8 (42.1)	3 (12.5)	0 (0.0)	0 (0.0)	
	Several groups	2 (10.5)	9 (37.5)	1 (100)	3 (30.0)	
	Many groups	3 (15.8)	10 (41.7)	0 (0.0)	7 (70.0)	
CD20	Absent	13 (68.4)	5 (20.8)	1 (100)	0 (0.0)	<.001
	Occasional	5 (26.3)	9 (37.5)	0 (0.0)	4 (40.0)	
	Several groups	1 (5.3)	3 (12.5)	0 (0.0)	0 (0.0)	
	Many groups	0 (0.0)	7 (29.2)	0 (0.0)	6 (60.0)	
CD68	Absent	14 (73.7)	0 (0.0)	0 (0.0)	1 (10.0)	<.001
	Occasional	1 (5.3)	2 (8.3)	0 (0.0)	0 (0.0)	
	Several groups	3 (15.8)	3 (12.5)	1 (100)	0 (0.0)	
	Many groups	1 (5.3)	19 (79.2)	0 (0.0)	9 (90.0)	
CD8	Yes	3 (15.8)	4 (16.7)	0 (0.0)	1 (10.0)	1.000
	No	16 (84.2)	20 (83.3)	1 (100)	9 (90.0)	

Table 4

Association between the degree of microcalcification and markers of inflammatory cells among the operated patients (N = 54).

Variables		Degree of microcalcification				P-value
		Absent (%)	Trace/mild (%)	Moderate (%)	Severe (%)	
CD3	Absent	6 (31.6)	1 (5.9)	0 (0.0)	1 (10.0)	.005
	Occasional	8 (42.1)	1 (5.9)	1 (12.5)	1 (10.0)	
	Several groups	2 (10.5)	5 (29.4)	5 (62.5)	3 (30.0)	
	Many groups	3 (15.8)	10 (58.8)	2 (25.0)	5 (50.0)	
CD20	Absent	13 (68.4)	1 (5.9)	2 (25.0)	3 (30.0)	.016
	Occasional	5 (26.3)	7 (41.2)	3 (37.5)	3 (30.0)	
	Several groups	1 (5.3)	2 (11.8)	1 (12.5)	0 (0.0)	
	Many groups	0 (0.0)	7 (41.2)	2 (25.0)	4 (40.0)	
CD68	Absent	14 (73.7)	0 (0.0)	0 (0.0)	1 (10.0)	<.001
	Occasional	1 (5.3)	0 (0.0)	1 (12.5)	1 (10.0)	
	Several groups	3 (15.8)	2 (11.8)	1 (12.5)	1 (10.0)	
	Many groups	1 (5.3)	15 (88.2)	6 (75.0)	7 (70.0)	
CD8	Yes	3 (15.8)	3 (17.6)	1 (12.5)	1 (10.0)	1.000
	No	16 (84.2)	14 (82.4)	7 (87.5)	9 (90.0)	

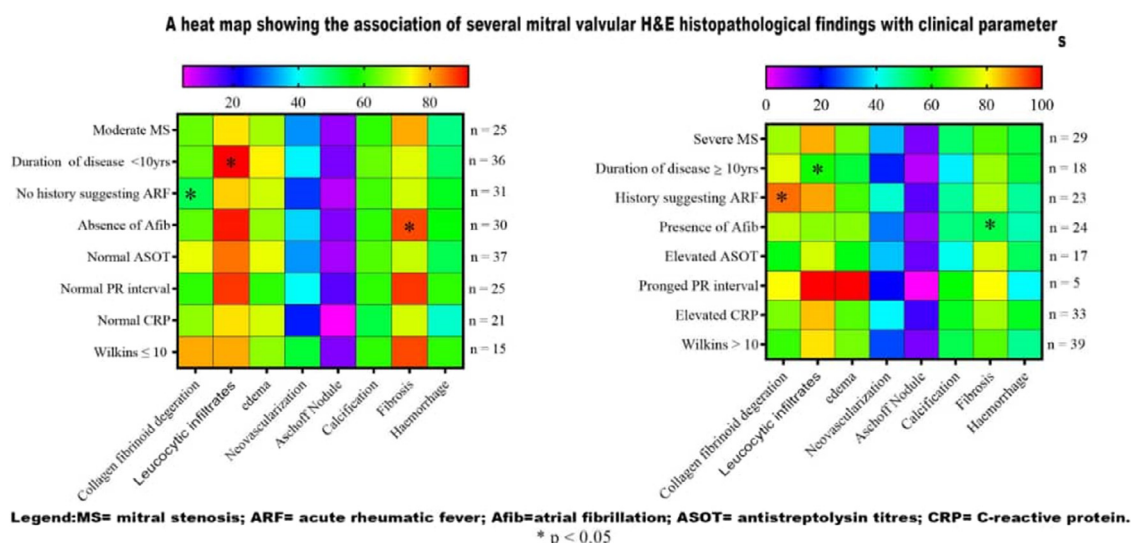


Fig. 3. A heat map showing the association of several mitral valvular H&E histopathological findings with clinical parameters. ARF = acute rheumatic fever; Afib = atrial fibrillation; ASOT = antistreptolysin titre; CRP = C-reactive protein; MS = mitral stenosis.

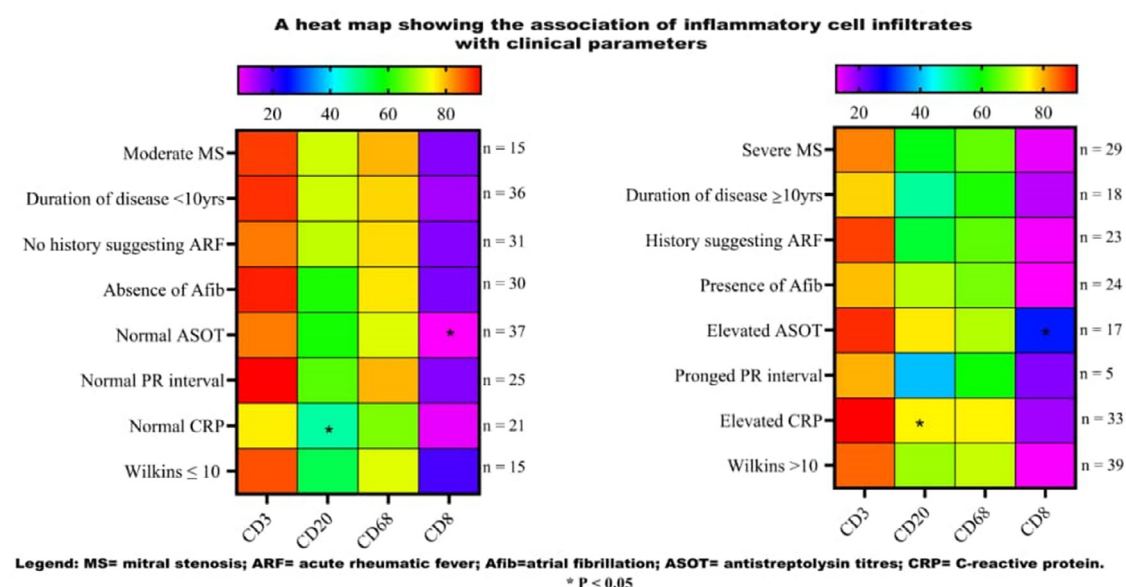


Fig. 4. legend. A heat map showing the association of inflammatory cell infiltrates with clinical parameters. ARF = acute rheumatic fever; Afib = atrial fibrillation; ASOT = antistreptolysin titres; CRP = C-reactive protei.

Fig. 4 depicts a statistically significant difference in presence of: CD20 staining cells among patients with normal vs elevated CRP (47.6% vs 75.8%, $P=0.035$) and CD8 staining cells among patients with normal vs elevated ASOT (8.1% vs 29.5%, $p=0.041$). The remaining CD staining cells did not show a statistically significant association with the analysed parameters.

4. Discussion

The present single-centre prospective study interpreted the histological findings observed in surgically excised rMS valves and explored how those findings correlate to the clinical presentation, disease pathogenesis and management. Our study showed higher MV calcium levels in individuals of >30 years, male, and with higher trans-MV pressure gradients. The degree of inflammatory cell infiltrates was associated with the extent of valvular calcification and the degree of microcalcification. ARF and shorter duration

of disease were associated with the increase in cells of acute inflammation.

The median (range) age of the patients of 39 (14– 57) years and the female 34 (63%) predominance is similar to other studies which reported RHD to be more common in females than males in the ratio of 2:1 [2,10,11,35]. In Africa, rheumatic mitral stenosis presents at an early age with rapid progression and severe disability at an early age [36]. The reported rapid progression was also observed in our study and could partly be explained by the fact that patients with a shorter duration of disease already had higher amount of valve calcification. Also, our study showed that a shorter duration of disease was associated with the increase in cells of acute inflammation. The higher amount of valve calcification and an increase in cells of acute inflammation among patients with a shorter disease duration in developing countries could be due to some reasons. First, factors predisposing to ARF persist and penicillin prophylaxis is usually inadequate [37–39]. Second, although secondary antibiotic prophylaxis reduces a risk of disease progres-

sion [40], many RHD patients fail or lack to adhere to secondary prophylaxis [35,38,39]. Neo-angiogenesis, calcific deposits, and inflammation all of which occur at a shorter disease duration play a role in the formation of valvular calcification [7,12].

Our study showed that with H&E staining, 37 (68.5%) specimens had fibrinoid degeneration, 44 (81.5%) leucocytic infiltrates, 37 (68.5%) edema, 18 (33.3%) neovascularization, 6 (11.1%) Aschoff nodules, 30 (55.6%) interstitial calcification, 39 (72.2%) fibrosis and 28 (51.9%) revealed fresh haemorrhage. These findings are similar to those reported by Rashed et al [16] and Suresh et al [8] implying a similar pathogenesis. The current study revealed moderate to severe mitral valve calcification with von Kossa stain in 18 (33.3%) specimens, slightly similar to that observed by Chopra et al [27] which was 36.4%.

Our study revealed a higher MV calcium level among males than females, patients with >30 years than with ≤30 years and those with higher trans-MV pressure gradients. Similarly, other studies reported that stenotic MV calcium occur more frequently and in huge amounts in men than women, in older than younger individuals, and in patients with severe MS [9,10].

In this study, there was a fair agreement between echocardiography and von Kossa stain (gold standard) for valvular calcification detection. Valvular calcium is a known predictor of poor outcomes after percutaneous balloon mitral valvuloplasty (PBMV) in both immediate and long term [10,11,41]. In circumstances where echocardiography is not confirmatory, fluoroscopy or computed tomography could be used to assess the severity and location of calcification. Alternatively, other methods for predicting outcomes following PBMV apart from Wilkin's score [42] can be used, for example, the echocardiography score revisited [43] and the Cormier score [44].

Similar to previous studies [7,8,12], our study showed a highly statistically significant association between the extent and distribution of valvular calcification with the severity of inflammatory cellular infiltrates. These inflammatory cells, calcific deposits and neovascularization has been postulated to be involved in calcification formation. Ambari et al [45,46] have recently reported the role of angiotensin converting enzyme inhibitor ramipril in the reduction of fibrosis in rMS. In RHD, fibrosis is induced by Angiotensin II through the stimulation of transforming growth factor- β , which eventually increases the binding of interleukin-33 to a soluble decoy receptor instead of its natural receptor. The overall effect is upregulation of Angiotensin II and progression to fibrosis.

As previously reported by other researchers [7,12], this study showed that the extent of valvular calcification and degree of microcalcification has a strong association with the severity of inflammatory cellular infiltration. As postulated [47–49], there is a resemblance between diseases involving lipid deposition, inflammatory cell infiltrations and calcification with that of atherosclerotic diseases. Indeed, as reported by Soini et al [50], patients administered with statin medications revealed a significantly lower tendency for neo-vessels formation.

Previous studies have shown that in ARF and patients with active RHD, there is increased production of proinflammatory cytokines [18] and these co-occurs with increased numbers of CD4⁺, CD8⁺, and CD25⁺ cells [6,21,22]. Interestingly, in the current study C-reactive protein and anti-streptolysin titres were statistically significantly high in both CD20 and CD8 staining cells but none of the tissues stained with markers for CD4 and CD25.

In developing countries like Tanzania, patients' selection and the type of valvular surgery are among the important factors to be considered because patients come late with complications of the disease. There are no local guidelines in our setting that could probably fit our patients' presentation with treatment outcomes. Ideally, there should be no active inflammatory process when these

patients are sent for surgery. However, it is challenging to make a diagnosis of ARF in areas where causes of fever and/or joint pain/swelling are plenty and therefore high expertise clinical suspicion complemented by appropriate laboratory investigations is needed.

Our study showed that secondary prophylaxis against recurrent attacks of ARF was given in 7 (13%) patients. Unfortunately, of the 2 (3.7%) patients diagnosed to have ARF, none of them had received the prophylaxis despite being eligible. Appropriate use of secondary prophylaxis is a cost-effective approach for preventing morbidity and mortality associated with ARF [51]. Low uptake of benzathine penicillin G has been documented in several countries [35,38,39] highlighting the need to identify barriers and enhance its access within the framework of care for chronic diseases in countries affected by RHD. As part of the management, after the histopathological results we prescribed a long-term acetylsalicylic acid 75 mg once daily and benzathine penicillin G injection 2.4 MU monthly to this study cohort as our local protocol.

4.1. Strengths and limitations of the study

Our study was conducted at the country sole hospital performing valvular surgeries making the study a representative of the country. The small sample size of this study limited further analysis. However, by utilizing three advanced histopathological techniques, important analyses were attained.

5. Conclusions

This study confirms that high MV calcium are found in patients who are old, male, and with severe mitral stenosis and that valvular calcification is associated with cellular infiltration. We found a low rate of secondary prophylaxis and two patients with ARF. Our findings are comparable with those from other countries suggesting similar pathogenesis and thus intervention modalities. We recommend: i) rigorous preoperative workup to rule out active inflammation, ii) increased uptake of secondary prophylaxis for recurrent attacks of acute rheumatic fever, and iii) the use of anti-inflammatory and antibiotic prophylaxis postsurgery.

Ethics approval statement

This study was approved by the institutional review board of the Muhimbili University of Health and Allied Sciences

Authors' contributions

Conceived and designed the study: RKM, PC, SC, AK; Data collection: RKM, MB, AN, AM; Analysed the data: RKM, AM, AN; RKM wrote the first draft of the paper and subsequent drafts in collaboration with PC, AM, MJC, GK, AK, LF and SC. All authors have given final approval of the version to be published.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.carpath.2022.107434](https://doi.org/10.1016/j.carpath.2022.107434).

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