Med. J. Cairo Univ., Vol. 62, No. 1, March: 101 - 112, 1994

Right Ventricular Myocardial Factor in Chronic Rheumatic Heart Disease

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Abstract

Right ventricular myocardial factor (RVMF) has not been adequately examined in patients with rheumatic heart disease (RHD). 31 patients (pts) with RHD and absent rheumatic activity were examined by endomyocardial biopsy and RV angiography. RV ejection fraction (RVEF) was normal in 22 (70%), decreased in 5 (16%) and borderline in 4 (12%). Evidence of active myocarditis was present in 16 (52%), resolving myocarditis in 6 (20%), while the rest showed healed or no myocarditis. Microvascular ischaemia was present in 10 (32%); all had active or resolving myocarditis. Increased amounts of intracellular glycogen and interstitial glycosaminoglycans were detected in the majority of pts. None of the pathologic changes correlated to RVEF. Pathologic evidence of RVMF was present in more than 50% of pts, yet was insignificant as far as RVEF is concerned.

Introduction

RHEUMATIC heart disease with its valvular sequelae constitute a major health problem in Egypt and other developing countries. Valvular affection by the rheumatic pathology poses a hemodynamic burden on the right ventricle by inducing pulmonary hypertension thus raising its afterload. Although previous studies pointed out clearly to the presence of an inverse relation between right ventricular ejection fraction and pulmonary hypertension in valvular heart disease [1], others demonstrated that right ventricular systolic dysfunction can occur in the absence of pulmonary hypertension and that its systolic function can be maintained in some cases despite the presence of pulmonary hypertension [2].

The presence of a myocardial factor secondary to an ongoing chronic rheumatic myocarditis affecting right ventricular intrinsic contractility plays possibly an important role in right ventricular dysfunction in rheumatic heart disease. Such an ongoing myocarditis process has been postulated to be a contributing factor to left ventricular dysfunction in this disease although proof to its presence remained controversial. Affection of right ventricular myocar-

dium by a myocarditis process would have definitely an additive effect to the hemodynamic burden imposed by long standing pulmonary hypertension. No solid proof for its existence was reported however, in the literature till present, and this factor needs further clarification.

Patients and Methods

This study included 31 patients with chronic rheumatic valvular heart disease. They were selected from those attending outpatient clinics of the National Heart Institute, Cairo, Egypt. Their age ranged between 14 and 40 years (mean 26.6±6.3 years). 17 patients were males and 14 were females. All patients required to have a definite history of recurrent attacks of rheumatic activity to be included. All of them were also required to be in sinus rhythm, those with atrial fibrillation were excluded as this is a handicap for precise estimation of right ventricular volumes and systolic function. Other exclusion criteria included:

- Failure to obtain adequate right ventricular angiogram or right ventricular muscle biopsy.
- Concomitant clinical or laboratory evidence of rheumatic activity.
- Patients with systemic illness likely to affect cardiac function and those receiving vasodilator therapy.

Patients were subjected to clinical evaluation, resting electrocardiograms, Echo-Doppler studies and laboratory tests to detect valve lesions and exclude rheumatic activity. Cardiac catheterization and right ventricular angiography were then done for each patient followed by right ventricular endomyocardial biopsy.

Right Ventriculography:

After taking pressures from the right heart. Single plane right ventriculography was performed for every patient in the 30 degrees right anterior oblique position. A pigtail catheter was introduced into the mid right ventricular cavity through the tricuspid valve taking care to avoid induction of ventricular arrhythmia. Injection of contrast material (Renographin 76%) was carried out by a pump injector using a dose of 12-15 cc/sec over 3 to 4 seconds for a total dose of 30-60 cc. One centimeter scale metal grid was then filmed at the mid-chest level for calibration purposes. After film processing, end systolic and end diastolic frames of at least three cardiac cycles were planimetered taking care to use frames at which the right ventricle was completely opacified disregarding minor dropouts of the outline due to trabeculation. Frames of extrasystolic and postextrasystolic beats were discarded from the calculations. Right ventricular end systolic and end diastolic volumes were then calculated according to the area-length formula described by Ferlinz [3,4] for calculation of right ventricular volumes in the 30 degrees right anterior oblique projection according to this formula:

$$\text{RV volume} = \frac{0.4\ (\text{A RAO})^2}{\text{L RAO}} + 3.9$$

Where:

A RAO: Planimetered area in 30 degrees right anterior oblique.

L RAO: Long axis of right ventricular cavity in the same projection connecting the mid point of the pulmonic valve to the mid point of right ventricular base.

Right ventricular ejection fraction (RBEF) was then calculated according to the formula:

$$RVEF = \frac{\text{RV end diastolic volume - RV end systolic volume}}{\text{RV end diastolic volume}} = 3.9$$

Endomyocardial Biopsy:

At the end of cardiac catheterization, right ventricular endomyocardial biopsy samples were obtained using the technique described by Mason and Billingham [5]. The samples were obtained using kimal 104 cm teflon coated bioptome using the femoral approach technique. 3 samples were taken from the right side of the ventricular septum in each case.

Pathologic Studies:

The specimens obtained by the endomyocardial biopsy were immediately fixed in 10% buffered formalin solution. They were embedded in paraffin according to the standard procedures to make paraffin blocks. Slices of 5 micron thickness were prepared from paraffin blocks using a microtome. An average of 3 serial sections per slide were stained by the following stains:

A- Hematoxylin and Eosin Staining:

With this staining procedure, the following parameters were specifically looked for:

1- Myocardial fiber and nuclear sizes were estimated using an eye piece micrometer. The average of 100 cell counts was taken at power x 640 (Mikrometer Okularowy Siatkomy 019 10/10).

2- State of cardiac muscle fiber and the presence of degeneration, necrosis, or other cytoplasmic and nuclear changes.

3- Presence of interstitial inflammatory cellular infiltrations, oedema, or hemorrhage. This was semiquantitatively assessed as mild (+), moderate (++), or severe (+++) according to Edwards [6].

4- Number of small blood vessels and the presence of microvascular thrombi or vasculitic changes.

5- Presence of mural thrombi adherent to right ventricular endocardium.

6- Presence and rough quantitation of interstitial fibrosis.

B- Hematoxylin Stain:

This was used to demonstrate lipofuscin perinuclear granules (wear and tear pigment of cardiac muscle) which stain yellow brown by this stain.

C- Periodic Acid-Schiff (PAS) Stain:

This stain is a fundamental stain for carbohydrate histochemistry. It is used to demonstrate intracytoplasmic or interstitial polysaccharides and glycogen. Glycogen, glycolipids, neutral mucopolysaccharides and some non-sulphated mucosubstances give positive reactions with PAS and stain magenta (dark pink) in color [7]. In addition, glycoproteins such as those of collagen and reticulin as well as basement membranes are strongly PAS positive as they contain carbohydrate complexes conjugated to the protein moieties [8].

D- Toluidine Blue Stain:

This stain detects sulphated mucopolysaccharide groups as they stain metachromatically by giving it a purple color. This group of substances is characterized by their intense alcoholresistant property as contrasted to non-sulphated carbohydrates. The most important acid mucopolysaccharides detected by this stain are known as sulphated acid glycosaminoglycans (Sulph. AGAGs).

E- Alcian Blue Stain:

This was used to detect acid mucopolysaccharides of the ground substance and

the cells. It is particularly useful for detection of the group of substances known as acid glycosaminoglycans (AGAGs). Staining at pH of 2.5 was undertaken. By this method, the acid glycosaminoglycans were stained blue while the nuclei were stained red.

F- Verhoff Van-Gieson (VVG) Stain:

This stain was used to demonstrate various fibrillary components as it colors the elastic tissue black, the collagen fibers red and the muscle fibers yellow. It has been advocated by Fenoglio and Marboe [9] as an appropriate stain for specific demonstration of collagen in cardiac muscle biopsies.

G- Reticulin or Silver Stain (Gordon-Sweets Stain):

The tissues stained by this stain appear black in color. It was used to demonstrate reticular framework (type III collagen) of the stroma [10].

H- Congo-Red Stain:

This stain was used to demonstrate stromal amyloid and amyloid-like material which give an orange red color on staining.

Control Specimens for Histopathologic Studies:

Control specimens were obtained from 5 postmortem hearts of subjects who died out of non-cardiac diseases with no history of cardiovascular abnormalities during life. These specimens were fixed in 10% buffered formalin and were subjected to the same fixation and staining procedures applied to the specimens of patients. The post-mortem specimens served as control slides for comparison with the changes observed in specimens of the patients.

Statistical Methods:

An IBM compatible PC was used to store and analyze the data and to produce graphic presentation of some important results. The statistical packages Microstat and SAS were used for the statistical analysis. Standard statistical methods were used. Results are expressed as mean ± 1 SD. A p value of < 0.05 was considered statistically significant.

Results

The study included 31 patients; 17 males and 14 females ranging in age from 14 to 40 years (mean 26.6±6.3 yrs). Three subgroups of patients were identified; mitral valve disease (MVD) group (8 patients), aortic valve disease (AVD) group (8 patients) and combined aortic and mitral valve disease (CVD) group (15 patients). Pulmonary hypertension defined as pulmonary artery systolic pressure (PASP) exceeding 30 mmHg was present in 27 patients (87%). Of these, 18 patients had mild degree of pulmonary hypertension (defined as PASP between 31 and 50 mmHg) (9 patients with CVD, 5 with isolated MVD and 4 with isolated AVD). Moderate degree of pulmonary hypertension (defined as PASP between 51 and 70 mmHg) was present in 4 patients; all belong to the MVD subgroup. Severe pulmonary hypertension (defined as PASP over 70 mmHg) was present in 5 patients (3 with MVD and 2 with CVD).

Angiographic estimation of right ventricular volumes and ejection fraction showed that right ventricular end diastolic volume index (RVEDVI) exceeded the upper limit of 93 ml/m^2 (Fcrlinz, 77) in 22 patients. Mild RV dilatation (RVEDVI 95 to 125 ml/m^2) in 11 patients, moderate right ventricular dilatation (RVEDVI from 125 to 150 ml/m^2) in 4 patients and severe dilatation (RVEDVI > 150 ml/m^2) in 7 patients. Right ventricular end systolic vol-

ume index (RVESVI) exceeded the upper limit of 50 ml/m^2 in 21 patients.

Estimation of angiographic right ventricular ejection fraction (RVEF) showed that it was lower than lower limit of 56% (Ferlinz, 77) for single plane area-length method in 5 cases (two with CVD, two MVD and one case with AVD), borderline low levels were present in additional 4 patients (3 with CVD and one with MVD) while normal RVEF was present in 22 patients.

Pathologic Results:

According to cellular and interstitial changes, the patients could be categorized into three groups:

1- Active Myocarditis Groups:

Evidence of active myocarditis was present in 16 patients (52%). Eight of these patients had double valve lesions (CVD), 5 had AVD and 3 MVD. In this group cellular damage in the form of degenerations and/or necrosis was demonstrated as follows: 5 cases of cloudy swelling, one case of vacuolar degeneration, 5 cases of hyaline degeneration, one case of fatty degeneration and 4 cases of cellular necrosis (sarcolysis). Nearby myocytes were normal, or displayed atrophy or less commonly hypertrophy. In addition to evidence of cellular damage, all cases showed cellular infiltration by round cells and/or polymorphonuclear leucocytes in the interstitial tissues in the regions adjacent to areas of cellular damage. Interstitial oedema and/or hemorrhage was present in 13 of these patients. No specific lesion (e.g. Aschoff bodies) was detected in any case.

Additional features characteristic of this group included reticular framework disorganization in the majority of cases (all but three cases). AGAGs were a feature in the interstitium and were condensed around the individual muscle fibers inn areas of affection (inflammation). Evidence of patchy fragmentation of the sarcolemma by silver staining was detected indicating myocardial fiber damage. This was present with or without loss of nuclear staining and loss of cytoplasmic striation depending on severity of fiber damage.

2- Resolving Myocaditis Group:

This group included 6 patients (20%), 3 of them had MVD and 3 had CVD. It was characterized by absence of myocardial fiber damage (degeneration and/or necrosis) by routine staining (H and E). Muscle fibers were mostly of normal size or showed hypertrophy. Inflammatory cells were mild but still present. AGAGs were still demonstrable both as perisarcolemmal condensations and in the interstitium. Some fragmentation of the sarcolemma was still demonstrable. Silver staining of the nucleus was absent. Interstitial fibrosis was evident in addition to disorganization of the reticular framework in some of the cases.

3- Healed Myocarditis Group:

Healed myocarditis was detected in 9 cases in which there was excessive replacement fibrosis with no or scanty cellular infiltrate and no evidence of active myocardial fiber damage (degeneration or necrosis) by routine light microscopy stains. Histochemically, disorganization of the reticular framework was present in two cases. Fragmentation of sarcolemma was still observed but to a lesser extent and absence of nuclear staining with silver stain was evident. AGAGs were not demonstrable except in one case.

The aforementioned three groups shared an abnormal increase in sulphated AGAGs and patchy cytoplasmic proteogly-

cans and/or glycogen. No amyloid was observed and 8 cases showed lipofuscin increase. Interstitial oedema was observed in 12 cases.

Evidence of microvascular ischaemia was observed in 10 cases all had active or resolving myocarditis. 6 had CVD, 2 had MVD and 2 had AVD. The diagnosis of microvascular ischaemia was based on the presence of microthrombi in small myocardial blood vessels. In addition, vasculitis changes were evident in two patients and increased vascularity was detected in 4 more patients.

Statistical Methods:

Right ventricular myocardial pathologic changes were tested for statistical relations versus right ventricular ejection fraction. Pathologic changes that were tested included: evidences of myocarditis, microvascular ischaaemia and degenerations, as well as semiquantitative estimation of degree of myocardial fibrosis and accumulation of acid glycosaminoglycans (AGAGs) and sulphated AGAGs. In addition, fiber size in microns was correlated with RV ejection fraction. None of the pathologic parameters used correlated to RVEF.

Discussion

Although many aspects of myocardial dysfunction in rheumatic heart disease can be explained by chronic hemodynamic loads imposed on ventricular myocardium, the presence of continuous myocardial affection by an ongoing subclinical rheumatic process has been postulated since a long time [11,12]. Such "smoldering" rheumatic myocarditis was postulated to be responsible for left ventricular dysfunction in cases with isolated mitral stenosis who have no adequate hemodynamic explanation for this dysfunction. Although reduced left

Table (1): Angiographic Findings Among Patients.

No.	Valve disease	RVEDVI	RVESVI	RVEF
1	CVD	121	48	60
2	CVD			
3	CVD	125	48	61
4	CVD	177	91	48
5	CVD	99	42	57
6	AVD			
7	AVD	124	49	62
8		179	58	67
9	AVD	173	76	56
10	AVD	88	43	56
11	CVD	114	43	62
12	CVD			
13		200	131	34
14	AVD	139	49	65
15	MVD	186	113	39
16	MVD	115	46	60
17	CVD	170	51	70
18	MVD	142	55	60
19	CVD	52	20	62
20		120	54	55
21	AVD	107	42	60
22		146	60	59
23	AVD	161	83	48
24	CVD	100	34	65
25	MVD	115	50	56
26	AVD	133	47	65
27	AVD			
28	AVD	48	18	60
29	AVD			
30	AVD	94	33	65
31	MVD	100	30	69

* RVEDVI: Right ventricular end diastolic volume index (ml/m^2).

* RVESVI: Right ventricular end systolic volume index (ml/m^2).

* RVEF : Right ventricular ejection fraction.

* CVD : Combined aortic and mitral valve disease.

* AVD : Aortic valve disease.

* MVD : Mitral valve disease.

Table (2): Pathologic Classification of Patieents.

No.	Myocarditis	Microvasc. ischaemia	Cellular size
1	Healed	Absent	Normal
2	Healed	Absent	Atrophy
3	Active	Absent	Normal
4	Active	Absent	Hypertrophy
5	Resolving	Absent	Hypertrophy
6	Healed	Absent	Atrophy
7	Active	Absent	Atrophy
8	Healed	Present	Atrophy
9	Resolving	Present	Hypertrophy
10	Heled	Absent	Atrophy
11	Active	Absent	Hypertrophy
12	Active	Absent	Atrophy
13	Active	Absent	Normal
14	Active	Absent	Norml
15	Resolving	Absent	Hypertrophy
16	Healed	Absent	Atrophy
17	Healed	Absent	Hypertrophy
18	Active	Present	Atrophy
19	Resolving	Present	Hypertrophy
20	Active	Present	Normal
21	Healed	Present	Atrophy
22	Resolving	Absent	Hypertrophy
23	Active	Absent	Atrophy
24	Active	Present	Normal
25	Active	Present	Normal
26	Active	Absent	Hypertrophy
27	Active	Present	Hypertrophy
28	Active	Present	Atrophy
29	Healed	Absent	Atrophy
30	Resolving	Absent	Hypertrophy
30	Active	Absent	Hypertrophy

Table (3): Histopathology Results.

	Active	Resolving	Healed	Control
MF damage	Present			
Inflammation	+++	++	-/Scanty	
MF size	N/A > H	N/H	A	N
AGAGs (condens)	+++ (S&N)	++ (S&NS)	+ (S)	
Disorganized reticular frame	+++	++	+/-	
Fragmentation sarcolemma	Present	Present		
Cytoplasmic glycogen & others	Patchy ≠	Patchy ≠	Patchy ≠	Diffuse granular
Fibrosis		+	+++	
Ischaemic element	Present	Present		
Mural thrombus	Present	Present	Present	

Fig. (1): Longitudinally cut myocardial fibers from a case of active myocarditis displaying myocardial fiber necrosis, oedema and a mixed inflammatory infitrate. (Haematoxylin & Eosin x 400).

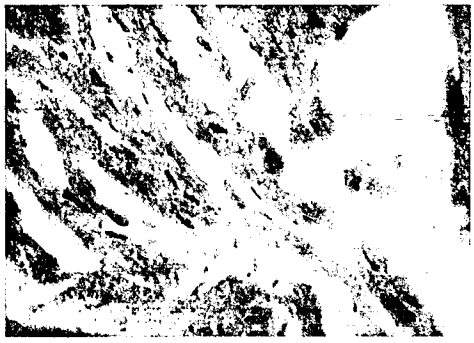

Fig. (2-a): Positive silver staining of nuclei, negative cytoplasm and incomplete sarcolemmal staining indicating fagmentation. (Gordon & Sweets x 400).

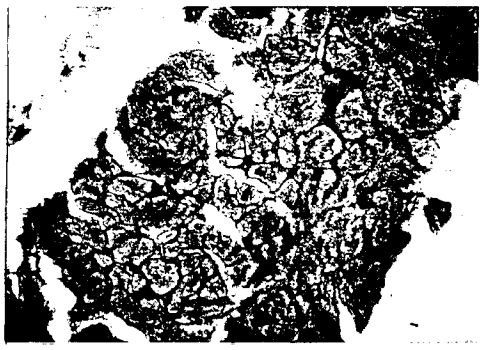

Fig. (2-b): Control specimen stained with silver stain showing positive nuclear, cytoplasmic and sarcolemmal staining. (Gordon & Sweets x 400).

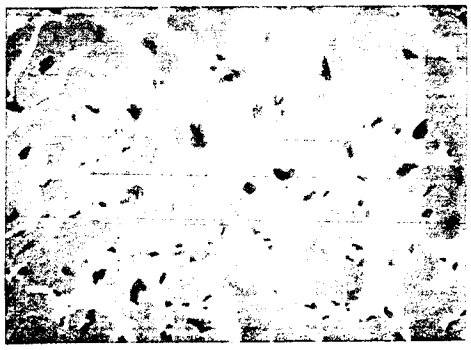

Fig. (3): Myocardium from a case of resolving myocarditis showing focal muscle fiber hypertrophy as evidenced by larger size of nucleus and increased cell size. (Haematoxylin & Eosin x 400).

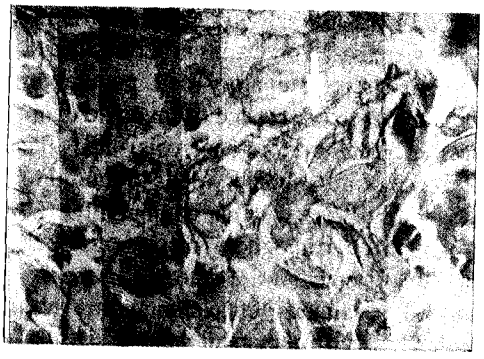

Fig. (4): Acid Glycosaminoglycan deposit in interstitial tissue (arrow) with a patchy perisarcolemmal distribution in a patient's sample. (Alcian Blue x 400).

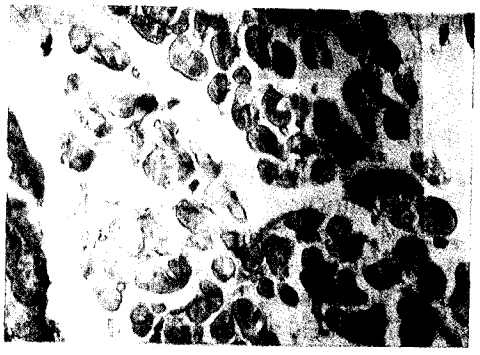

Fig. (5): Periodic Acid Schiff positivity of both sarcoplasm and sarcolemma and patchy sarcoplasmic negativity (arrow) in a patient's sample. (PAS x 200).

Fig. (6): Pink band of interstitial fibrosis (arrow) surrounded by dark yellow cardiac muscle fibers.
(Verhoeff Van Gieson x 400).

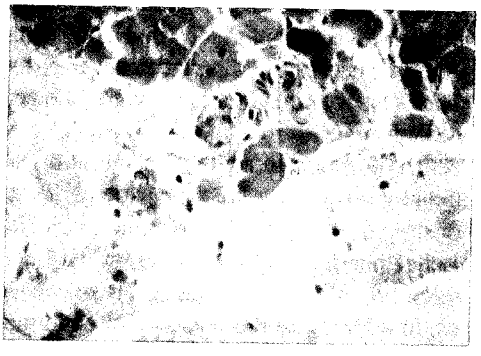

Fig. (7): Two interstitial blood vessels showing microthrombi. (Haematoxylin & Eaosin x 400).

ventricular preload was speculated by some to be responsible for this dysfunction, echocardiographic [13] as well as histopathologic [14] studies conducted in Egypt have pointed out clearly to the presence of primary myocardial affection in such cases. This conclusion has gained further support recently by the study of Lee and Lee [15] who proved with solid evidences the presence of ultrastructural changes involving the myofibrils, mitochondria, nuclei as well as other cytoplasmic and membranous elements of left ventricular myocardium in mitral stenosis patients. These changes were more extensive in cases with abnormal left ventricular function though they did not correlate with it. Further evidence was substantiated by the results of the work done by Marcus et al. [16] who demonstrated the presence of

an ongoing rheumatic carditis in 16% of patients undergoing mitral valve replacement.

By the use of endomyocardial biopsy (EMB) technique, we were able to prove unequivocally the presence of an ongoing right ventricular myocardial pathologic process in patients with rheumatic heart disease. In this study, we have attempted to apply this technique to study right ventricular myocardial affection by the rheumatic process. A variety of pathologic processes were demonstrated to affect right ventricular myocardium. These changes include the presence of subclinical active or resolving myocarditis of varying degrees of severity in 76% of our patients with 16 of them in the active phase and 6 in the resolving phase with resultant myocardial cell degenerations, necrosis and replacement fibrosis. The rest of our patients who showed healed myocarditis had also subtle evidence of injury. In addition, a subset of patients with myocarditis (10 patients) demonstrated the presence of microvascular ischaemia secondary to microthrombi and vasculitis in their specimens. These changes, in addition to the presence of abnormal amounts of metabolites such as glycosaminoglycans (GAGs), glycogen and glycoproteins, the observed interstitial changes and the abnormal silver staining reaction, were detected in a large number of specimens so that none of them were completely free.

According to Fenoglio and Marboe [9], the general basis on which myocarditis was histopathologically characterized was by inflammatory infiltrate directly associated with myocyte damage in the absence of coronary vascular disease. Active myocarditis or its resolving phase (borderline phase) were present in 76% of our patient population in the absence of clinical evidence of disease activity. Criteria of diagnosis were based on Dallas criteria [17] and depend on inflammatory cell population with evidence or absence of myocyte damage (degeneration or necrosis) and the presence of inflammatory cells of a mild to moderate degree with or without fibrosis. Although no pathognomonic lesions such as Aschoff bodies were detected in the examined specimens of patients, this was probably due to the small sample size obtained by endomyocardial biopsy technique in view of the known patchy distribution of these lesions. The presence of a definite history of recurrent rheumatic activity in all of these patients makes the possibility of a coincident myocarditis process of another etiology highly unlikely. In the absence of clinical and laboratory evidence of acute exacerbation of rheumatic activity, it seems logical to assume that these phases of myocarditis are expressions of a chronic myocarditis process. Such chronic myocarditis is probably responsible for the well recognized phenomenon of persistent moderate elevation of erythrocyte sedimentation rate in Egyptian rheumatic heart patients.

This study also documented the presence of a subgroup displaying microvascular ischaemia in right ventricular myocardium. These patients, whose age, history and examination exclude the presence of atherosclerotic coronary heart disease, displayed the presence of microthrombi in 10 of them in addition to vasculitis changes in another two. This microvascular affection was also supported by the presence of interstitial oedema as it reflects microvascular injury, possibly secondary to immunological injurious factor [18]. Such microvascular affection has been documented since long time in the myocardium

of patients with acute rheumatic carditis [19] and was assumed by some investigators to be an important factor in myocardial dysfunction in rheumatic heart disease [20]. The presence of microvascular ventricular ischaemia gets more importance in patients with pulmonary hypertension as in these cases there is a definite degree of subendocardial ischaemia that is proportional to the pressure overload. This subendocardial ischaemia was incriminated by some investigators to be the underlying cause of right ventricular dysfunction in patients with pulmonary hypertension [21]. It seems logical therefore to assume that the presence of microvascular affection in such patients is an additive factor to right ventricular dysfunction. It has to be pointed out that although the mere presence of microvascular ischaemia did not correlate with parameters of right ventricular systolic function, we did not have a quantitative method for measuring the degree of such ischaemia.

Acid glycosaminoglycans (AGAGS) and sulphated AGAGS were present in considerable amounts in the majority of our patients in the sarcoplasm and interstitial tissues while they were not detected in any of the control specimens. This may be the result of the associated inflammatory process or possibly reflect tissue damage caused by pressure overload.

In this study, there was a lack of statistically significant correlation between histopathologic findings of the patient group as a whole and various right ventricular systolic indices. Despite this, on individual basis, some of the patients such as cases number 4 and 23, showed depressed right ventricular ejection phase indices in the presence of near normal or mildly elevated levels of pulmonary artery pressures. Both patients displayed evidence of active myocarditis in their biopsy specimens and this was probably the underlying mechanism of depressed right ventricular systolic performance. Moreover, there was a trend towards more impairment of right ventricular systolic performance with severe degrees of fibrosis but this did not reach statistical significance. Such lack of correlation between histopathologic myocardial changes and indices of systolic function was demonstrated in several previous studies of left ventricular myocardial specimens [22,23].

That right ventricular systolic parameters did not correlate significantly in our study with most of the histopathologic changes is conceivable for many reasons. Some of these reasons are related to lack of parameters that purely reflect right ventricular contractility rather than its systolic performance which is highly affected by loading conditions. Other factors underlying such lack of correlation include the scanty amount of myocardium obtained by endomyocardial biopsy technique with possible sampling error and skipping of subepicardial changes [22], the semiquantitative nature of describing these pathologic changes and the lack of perfect criteria for classification of myocarditis to replace the currently used ones which are highly observer-dependent and do not help in patient prognostication.

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