IMAGES in PAEDIATRIC CARDIOLOGY

Right ventricular myocardium in Fallot's tetralogy: a light microscopic, morphometric and ultrastructural study

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Abstract

Aims:

To analyze peroperative biopsies of RV myocardium in Tetralogy of Fallot by light microscopy, morphometry and electron microscopy in order to determine the degree of hypertrophy and degenerative changes and to correlate these changes with clinical and haemodynamic parameters.

Materials and Methods:

Right ventricular myocardium obtained peroperatively during surgical correction of Tetralogy of Fallot along with age-matched control samples were processed for routine light and electron microscopy using standard processing techniques. Mean cell diameter was analyzed using manual morphometric methods and ultrastructural study was carried out using a Philips transmission electron microscope.

Results:

The most consistent features of hypertrophy were the mitochondrial changes and increased nuclear convolutions. Majority of the patients had hypertrophy with mild to moderate degenerative changes. Severe degeneration was associated with irreversibility and was related to the severity and chronicity of the disease. There was a significant correlation of the morphological changes with clinical and haemodynamic parameters.

Conclusions:

Peroperative histomorphometric and ultrastructural evaluation of the RV myocardium in Tetralogy of Fallot reflects the effect of haemodynamic stresses on the right ventricular muscle and correlates with clinical cardiac dysfunction. It may be a useful adjunct in determining the time for surgical intervention and in predicting clinical outcome.

MeSH: Heart defects, congenital, Tetralogy of Fallot, heart, Myocardium, ventricles, mitochondria

Introduction

It is a well known fact that congenital heart disease associated with right ventricular outflow tract obstruction leads on to right ventricular hypertrophy, and Fallot's tetralogy is one such entity. Persistence of hypertrophy leads on to progressive degeneration which may vary in grade, depending on the severity of obstruction and chronicity of the disease process.[1] Although there are extensive ultrastructural studies on acquired cardiac disease, particularly the cardiomyopathies,[2,3,4] similar studies on congenital heart disease[5,6] have been very limited in the literature.

In this study, the right ventricular myocardium of patients with tetralogy of Fallot (TOF) was peroperatively biopsied and studied using histomorphometry and electron microscopy. Particular attention was directed to the size, shape and pattern of arrangement of myocytes, intercalated discs, alterations in intracellular organelles, including mitochondria, nuclear abnormalities, degree of atrophy and interstitial changes including fibrosis. These morphological changes were categorized according to the criteria of Maron et al[7] as hypertrophic changes with or without degeneration, based on light microscopic and ultrastructural characteristics. The spectrum of morphological changes were correlated with clinical and hemodynamic parameters.

Materials & methods

This study was carried out on materials obtained from 12 patients with Tetralogy of Fallot, ranging in age from 8 months to 22 years (Figure 1), and 3 controls.

Figure 1 Age distribution
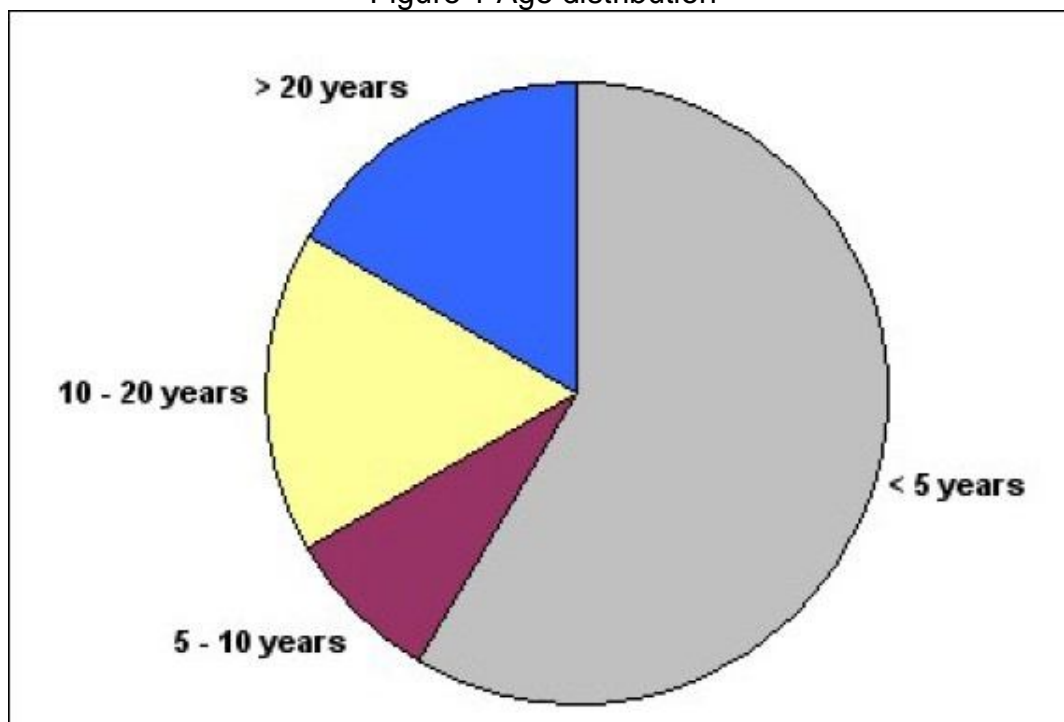
The patients underwent surgical correction following evaluation by echocardiography. During surgery, the obstructing right ventricular muscle was excised and a bit sent for sampling. In addition, control myocardial samples were obtained from normal hearts harvested for homograft valves from three brain dead individuals of similar age group with no known cardiac disease.

Sampling

Specimens of the right ventricular myocardium from patients of TOF were taken within 4-8 minutes of aortic cross clamping. Two samples were obtained of which one was fixed in 10% formalin for histomorphometric evaluation. The other sample was immediately minced into 1-2 mm size pieces, fixed for 12 hours in cold 3% glutaraldehyde in sodium cacodylate buffer and set aside for ultra-structural evaluation. Right ventricular myocardial samples obtained from controls were also fixed in a similar manner.

Histomorphometric study

After routine processing, the tissue was embedded in paraffin and 5-micron sections were cut. Haematoxylin and eosin stained sections were used for light microscopic examination and morphometric evaluation. Morphometry was done using an eyepiece micrometer and a slide graticule. In addition to cell size, light microscopic examination included study of alignment and orientation of muscle cells, orientation of myofibrils, nuclear changes, myocyte hypertrophy, atrophy, fibrosis and degenerative changes (table 1).

Table 1 Salient light microscopic features

S. No.	Age in Years	Size in Microns	Edema	Fibrosis	Loss of Myocytes	Defective Myocytes	Sclerotic Myocytes	Necrotic Myocytes	Cellular Atrophy
1.	1	16.26	Nil	Moderate	Nil	Few	Few	Nil	Nil
2.	2.5	19.15	Nil	Mild to moderate	Nil	Nil	Few	Nil	Nil
3.	8/12	16.55	Mild	Moderate	Nil	Nil	Few	Nil	Mild
4.	2.5	21.82	Nil	Mild	Nil	Nil	Few	Few	Mild
5.	2	27.9	Nil	Mild to Moderate	Nil	Few	Nil	Nil	Mild
6.	3	25.35	Focal	Moderate	Nil	Moderate	Nil	Few	Moderate
7.	3	21.7	Nil	Moderate to severe	Nil	Few	Nil	Few	Mild
8.	5	25.15	Nil	Mild to moderate	Nil	Moderate	Few	Few	Nil
9.	12	21.2	Mild	Moderate	Nil	Moderate	Moderate	Few	Moderate
10.	11	24.25	Nil	Moderate to severe	Moderate	Moderate	Nil	Nil	Nil
11.	22	23.7	Moderate	Mild	Moderate	Moderate	Nil	Moderate	Moderate
12.	20	27.95	Moderate	Moderate to severe	Moderate	Moderate	Few	Nil	Moderate

Mean myocardial cell diameter was calculated from 100 measurements of cross sections near the nucleus by the method described by Chalkley[8] and Arai.[9] (figure 2).

Electron microscopic study

Post-fixation of the glutaraldehyde fixed samples were carried out with cold 1% osmium tetroxide in Millonig's phosphate buffer for 3 hours. The tissues were then dehydrated in ethanol and propylene oxide and embedded in Maraglas epoxy resin. One-micron thin sections were stained with alkaline Toluidine blue. Ultra-thin sections obtained using a diamond knife were stained with uranylacetate and lead citrate and examined with a Phillips transmission electron microscope.

Figure 2 Histomorphometric evaluation of cell diameter vs. no. of cases
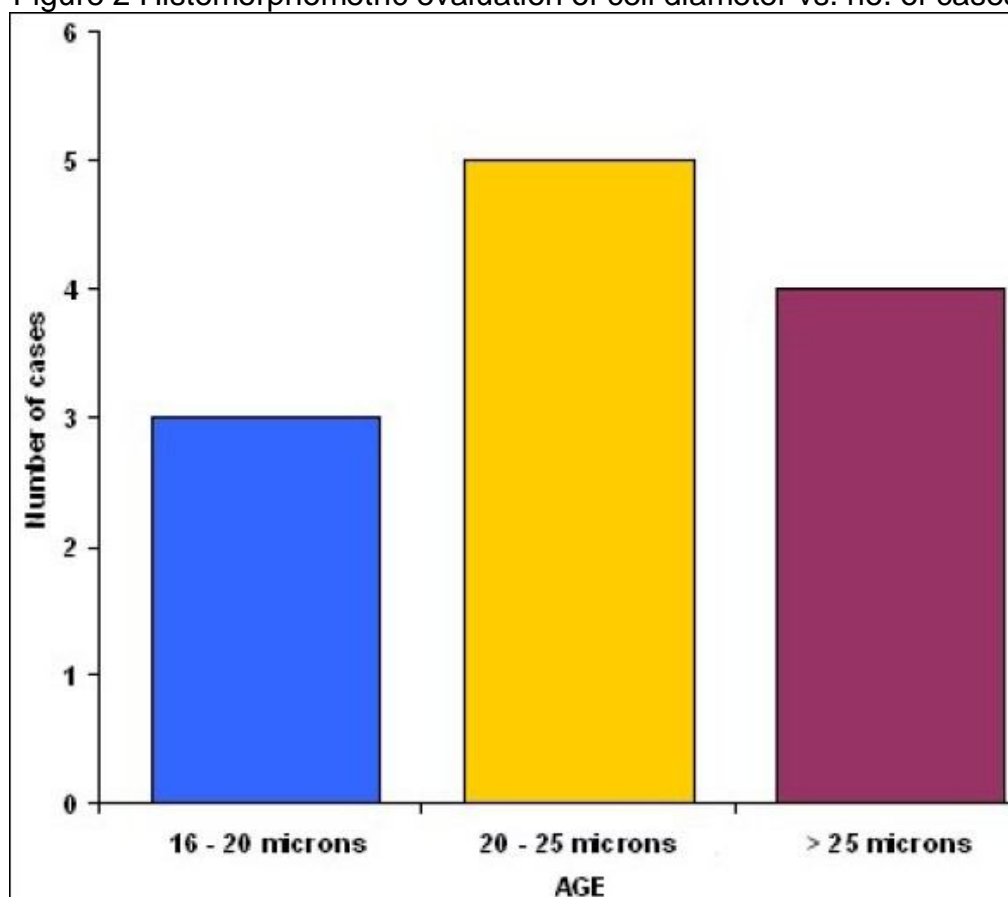
Clinical assessment

The following clinical assessements were carried out: evaluation for the presence of cyanotic spells, hematocrit, peripheral oxygen saturation, RVOT (right ventricular outflow tract) gradient, RVEDP (right ventricular end diastolic pressure), SPO2 (oxygen saturation) and PA/AO (pulmonary artery/aorta) ratio (table 2). All patients were evaluated by echocardiography. Cardiac cathetrisation was done in 11 patients. The severity of the RVOT obstruction was assessed by the gradient. All the patients underwent corrective surgery

for Tetralogy of Fallot under cardiopulmonary bypass with moderate hypothermia.

Table 2 Haemodynamic/Clinical parameters and relationship to nuclear changes and degree of fibrosis

S.No.	Age in years	Spells	HCT	SPO$_2$	RVEDP	RVOT Gradient	Fibrosis	Nuclear Convolutions
1.	1	Yes	39	79	7	70	Moderate	2+
2.	2.5	Yes	35	78	4	82	Mild to Moderate	1+
3.	8/12	No	32	83	5	51	Moderate	2+
4.	2.5	Yes	38	89	6	87	Mild	1+
5.	2	Yes	42	74	7	76	Mild to Moderate	2+
6.	3	Yes	45	76	4	78	Moderate	1+
7.	3	Yes	48	69	10	83	Mod to severe	3+
8.	5	No	64	55	7	79	Mild to Moderate	3+
9.	12	Yes	73	80	10	80	Moderate	1+
10.	11	Yes	64	80	9	76	Mod to severe	2+
11.	22	No	63	72	9	72	Mild	2+
12.	20	Yes	50	85	10	79	Mod to severe	3+

Hct=Haematocrit, SPO$_2$=Oxygen saturation, RVEDP=Right ventricular end diastolic pressure, RVOT gradient=Right ventricular outflow tract gradient

Results

Control samples of right myocardium were studied by light microscopy using routine H&E, semi thin sections which were stained by Toluidine Blue (figure 3) and by electron microscopy (figures 4,5) to study size, alignment, cross striations, organelles and nuclear contours (figure 6).

Figure 3 Control RV myocardium. Longitudinal section showing cross striations and normal nuclear contours. Semi thin section Toluidine blue LM X400
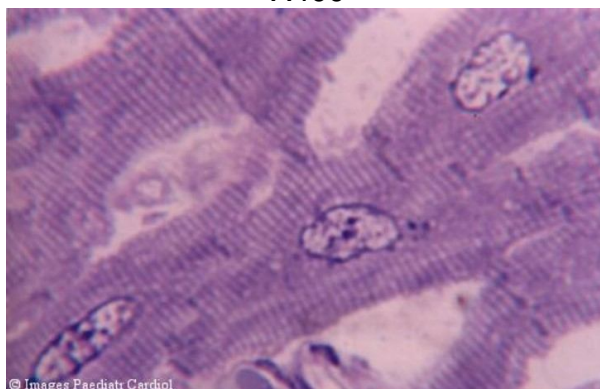
6

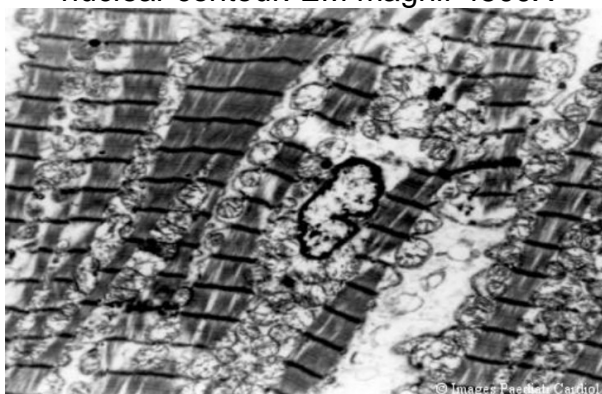Figure 4 Electron micrograph control RV muscle. Normal Z bands, smooth nuclear contour. EM magnif 4500X

Figure 5 Electron micrograph control RV muscle. EM magnif 30000X
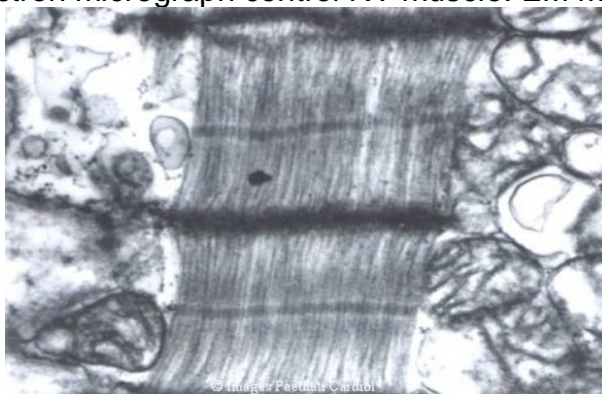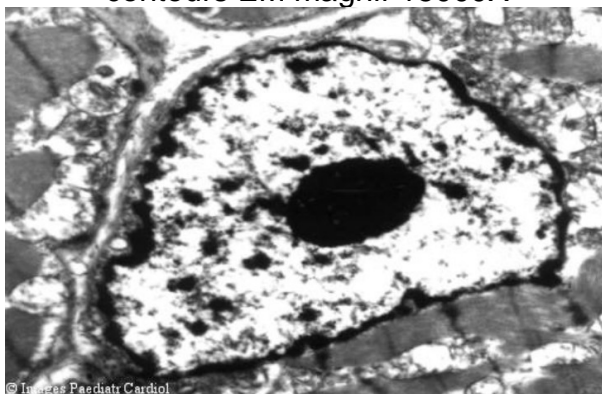

Figure 6 Electron micrographs control RV muscle. Note the smooth nuclear contours EM magnif 15000X

Histomorphometric studies

The principal light microscopic changes observed were cellular and interstitial The changes in myocardial cells of the patients were predominantly that of hypertrophy, oedema, myocytolysis (necrosis of myocardial cells), atrophy and sclerosis. The interstitium showed fibrosis, oedema and vascular dilatation.

Cellular changes

Presence of hypertrophy was considered unequivocal when the mean diameter of myocytes exceeded 20 microns as assessed by morphometry. Hypertrophy (figure 7) was observed in 9 out of the 12 cases studied.

In addition to hypertrophy, degenerative changes were observed characterized by varying degrees of myofibrillar disarray (figure 8).

In some cases, myocytes of 40 microns diameter were observed. Cellular atrophy of a mild to moderate degree was seen 8 cases (4 adults and 4 children). In severe degeneration atrophy of cardiomyocytes resulted in marked crowding of nuclei (figure 9).

Figure 7 Cross section TOF RV myocardium showing hypertrophy. Semithin section. Toluidine blue LM X400
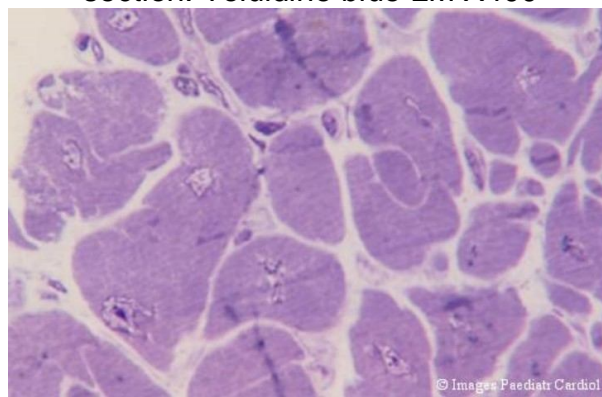

Figure 8 Longitudinal section TOF RV myocardium showing hypertrophy and myofibrillar disarray. Semithin section Toluidine blue LM X200
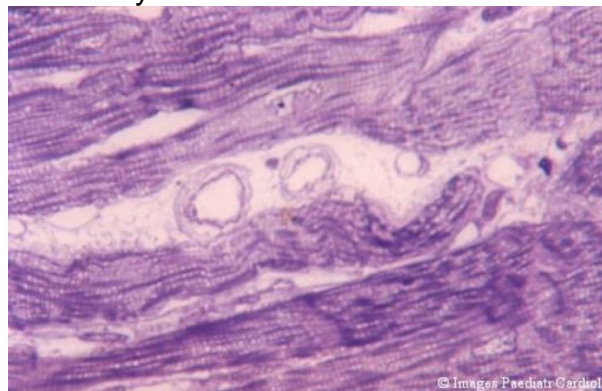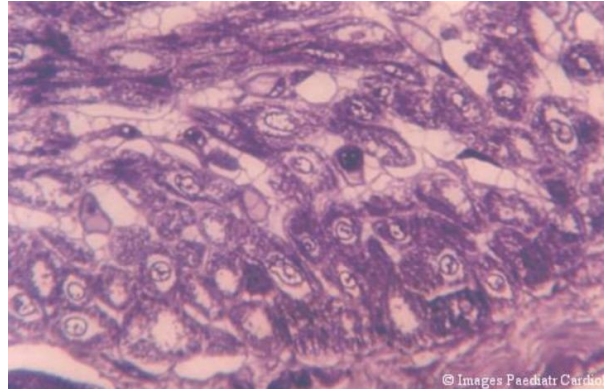
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Figure 9 CS TOF RV myocardium showing severe degeneration.The cardiomyocytes show marked crowding and atrophy. Semithin section Toluidine blue LM X200

Other cellular changes (oedema, myocytolysis and sclerosis) were observed in half of the cases. Z band abnormalities were also seen in association with varying grades of myofibrillar disarray (figures 10,11).
Defective myocytes were observed in 50% of the patients studied. Necrotic myocytes were predominantly seen in patients with a hematocrit of more than 60.

Figure 10 Longitudinal Section TOF RV myocardium showing hypertrophy, myofibrillar disarray and Z band abnormalities. Semithin section Toluidine blue LM X200
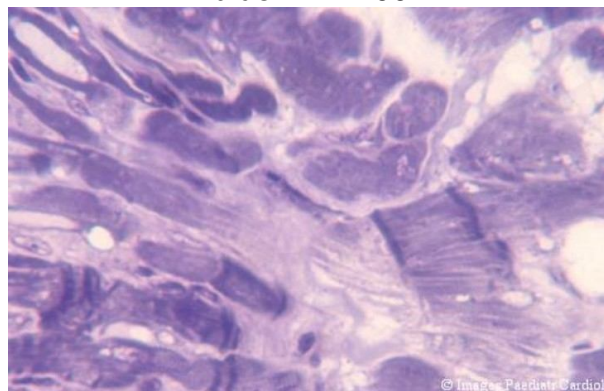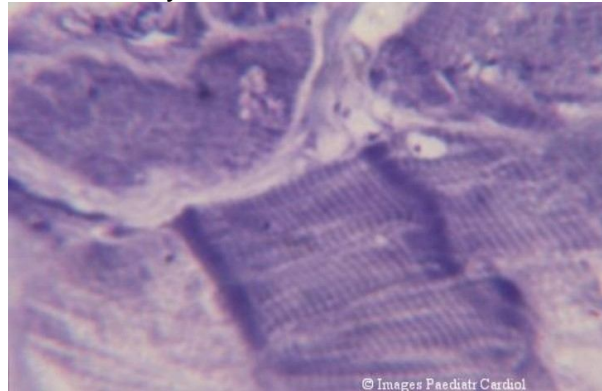
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Figure 11 Longitudinal Section TOF RV myocardium showing hypertrophy and myofibrillar disarray. Semithin section Toluidine blue LM X400

Nuclear changes

A prominent feature evident in semi thin sections of TOF RV cardiomyocytes were the nuclear abnormalities seen as elongation and increased irregularities and convolutions of the nuclear membrane, in addition to the above cellular abnormalities (figures 12,13).

Figure 12 Longitudinal Section TOF RV myocardium showing hypertrophy, myofibrillar disarray and increased nuclear convolutions. Semithin section Toluidine blue. LM X400

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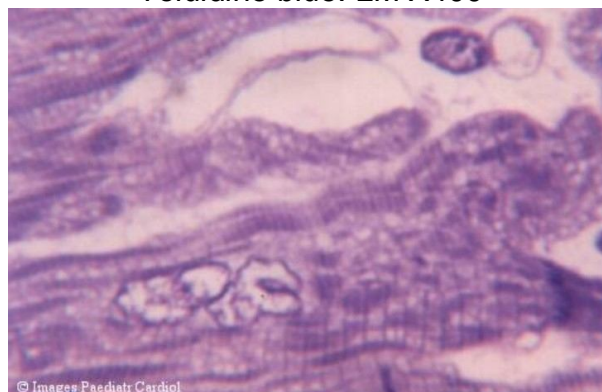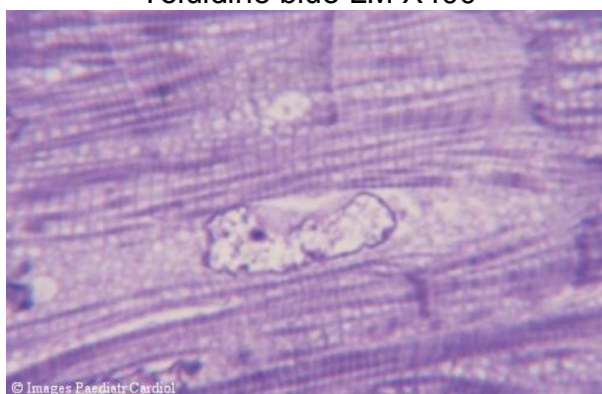
Figure 13 Longitudinal section: TOF RV myocardium showing hypertrophy, mild myofibrillar disarray, nuclear elongation and irregularity. Semithin section Toluidine blue LM X400

Interstitial changes

All the cases in this study had varying degree of fibrosis. The type of fibrosis may be described as focal, perimysial, plexiform or perivascular fibrosis. The types of fibrosis seen in majority of cases were either perimysial or perivascular. Three cases showed mild interstitial oedema, although it tended to be more prominent in the samples obtained from the adults. Moderate to marked increase in interstitial fibrosis (figures 14,15) was noted in at least 5 cases, the remaining showed only a mild/focal increase.

Figure 14 Longitudinal section TOF RV myocardium showing severe degeneration seen as marked interstitial and perivascular fibrosis. The cardiomyocytes appear pale with mild atrophic changes. Semithin section Toluidine blue LM X200

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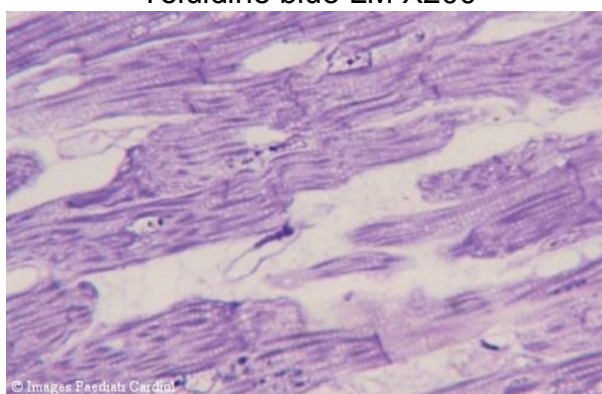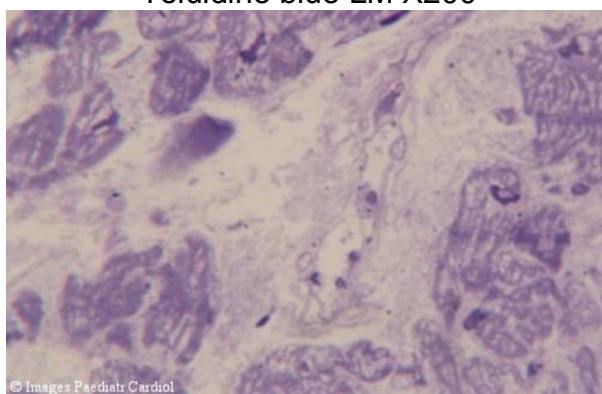
Figure 15 Cross section TOF RV myocardium showing hypertrophy, myofibrillar disarray with interstitial and perivascular fibrosis. Semithin section Toluidine blue LM X200

Electron microscopic changes

The electron microscopic changes were observed within the cytoplasm, organelles and nucleus (table 3).

Highlights of the cytoplasmic changes included myofibrillar disarray, Z band abnormalities in mild and moderate degeneration (fig 16) and intracytoplasmic vacuolations.

The principal organelles affected were the mitochondria. The sarcoplasmic reticulum showed proliferation and dilatation. In addition to these cellular changes, the interstitium and nuclei also showed significant alterations. The ultrastructural observations are as follows.

Table 3 Salient electron microscopic changes

S. No.	Age in years	Myofibrillar Disorganization	Intercellular Widening	Mitochondria	Myelin Figures	BM Thickening	Intracytoplasmic Vacuoles	Nuclear Size	Nuclear Convolutions
1.	1	Mild	Slight	Increased irregular	Few	Slight	Nil	Normal	Moderate
2.	2.5	Mild	Normal	Increased, irregular, ruptured	Nil	Nil	Nil	Increased	Mild
3.	8/12	Moderate	Marked	Increased, dilated	Few	Nil	Several	Increased	Moderate
4.	2.5	Mild	Slight	Increased, dilated	Nil	Nil	Nil	Increased	Mild
5.	2	Mild	Slight	Increase, irregular	Nil	Normal	Few	Increased	Moderate
6.	3	Moderate	Slight	Increased, irregular, ruptured	Nil	Nil	Several	Increased	Mild to Moderate
7.	3	Mild	Slight	Increased, irregular	Nil	Nil	Few	Increased	Severe
8.	5	Mild	Slight	Increased, irregular	Few	Nil	Few	Increased	Severe
9.	12	Moderate	Marked	Increased, dilated	Nil	Nil	Several	Increased	Mild
10.	4	Mild	Slight	Increased irregular	Nil	Nil	Nil	Increased	Moderate
11.	22	Moderate	Marked	Increased irregular	Nil	Nil	Several	Increased	Moderate
12.	20	Moderate	Moderate	Increased irregular	Moderate	Nil	Few	Increased	Severe

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Figure 16 Electron micrograph TOF RV myocardium showing moderate degeneration, increased mitochondria and myofibrillar disarray. EM magnif: 7000X
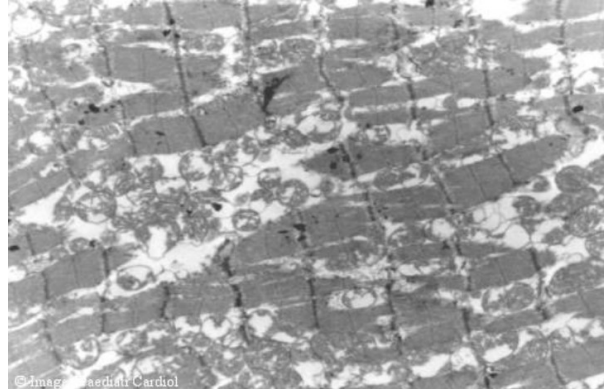

Cytoplasmic changes

Myofibrillar changes included reduction in the number (in 3 cases), disorganization including non-parallel arrangement in 6 cases, and focal lysis (in all cases). Intracytoplasmic vacuolation was seen to a marked degree in 4 cases (figure 17).

In addition, lipid vacuolation was seen in 2 cases. Myelin figures (figure 18) were seen in 4 cases - 3 children and 1 adult - and in the latter the myelin figures were more prominent.

Uncommon changes observed include lipofuscin granules and Z band abnormalities (1 case each).

Figure 17 Electron micrograph TOF RV muscle. Severe degeneration-intracytoplasmic vacuolation. EM magnif 3000X
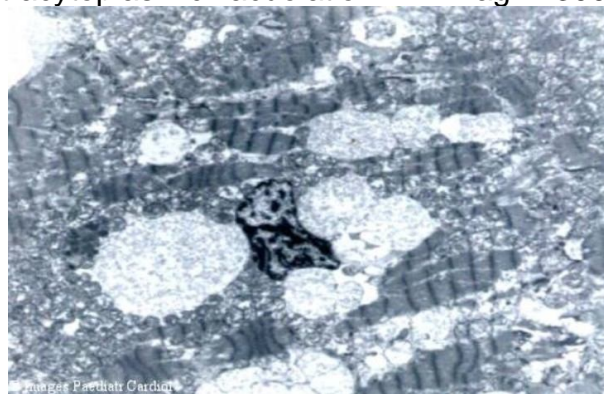
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Figure 18 Electron micrograph TOF RV muscle Severe degeneration- myelin figure (arrow) EM magnif 7000X

Changes in organelles

Changes in the mitochondria represented one of the important EM findings. There was a uniform increase in their number. In all cases they were irregular in shape and were dilated, and in a few, they appeared ruptured (figure 19). In 7 cases, there were small mitochondria.
Rough endoplasmic reticulum abnormalities were also seen (figure 20).

Figure 19 Electron micrograph TOF RV muscle. Markedly dilated, irregular and ruptured mitochondria. EM Magnif 15000X

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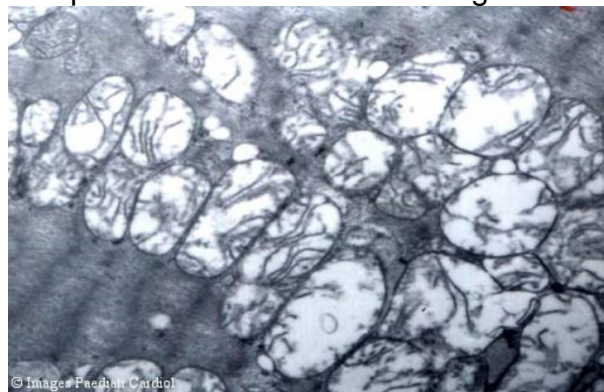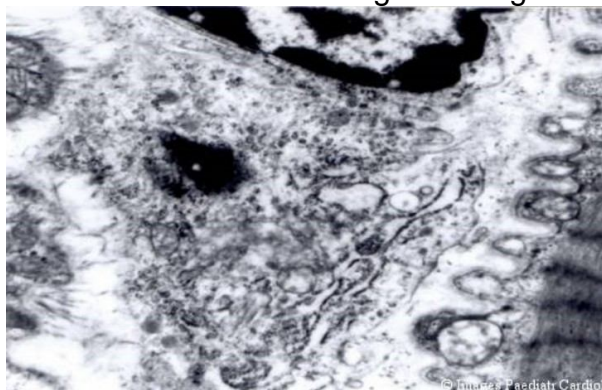
Figure 20 Electron micrograph TOF RV muscle. Severe degeneration- myelin figures, abnormal Z bands, rough endoplasmic reticulum abnormalities and basement membrane thickening. EM magnif 15000X

Intercellular junctions and basement membranes

Inter-cellular junction widening was seen in all except one. It was marked in 4 cases. (figures 21,22).
Multiple intercalated discs were evident in 2 cases. Intercellular dissociation was seen in majority of cases. Slight thickening of the basement membrane was seen in 2 cases.

Figure 21 Electron micrograph TOF RV muscle. Longitudinal section showing widened intercellular junctions (arrow). EM magnif 7000X
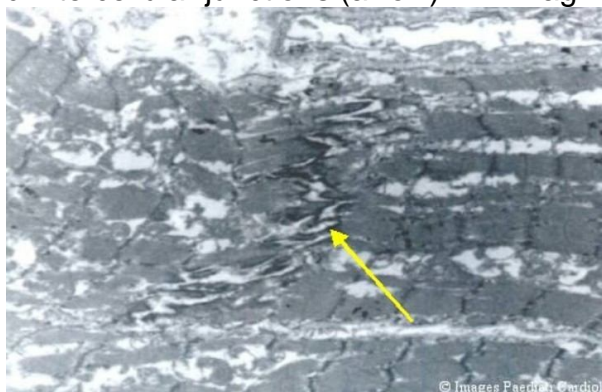

Figure 22 Electron micrograph TOF RV muscle. Longitudinal section: showing widened intercellular junctions (arrow) EM magnif 15000X

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Figure 23 Electron micrograph TOF RV muscle. Longitudinal section showing widened intercellular junctions (arrow). EM magnif 30000X

Nucleus

The nuclear size was increased in all cases, significantly so in 9 cases. There was elongation of the nucleus with moderate increase in nuclear convolutions (figures 24-26) which was evident in all the patients, but was marked in 3.

Figure 24 Electron micrograph TOF RV muscle. Hypertrophied cardiomyocytes, convoluted nuclei, abundant mitochondria. EM Magnif 7000X
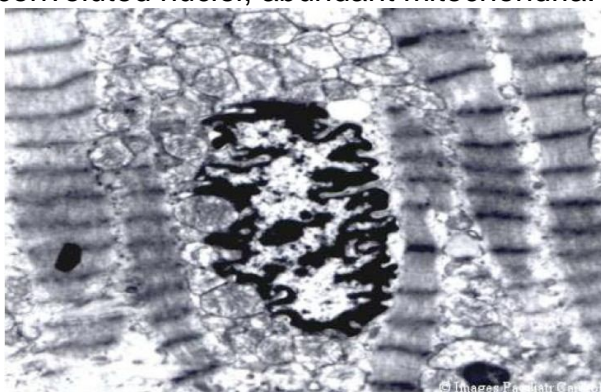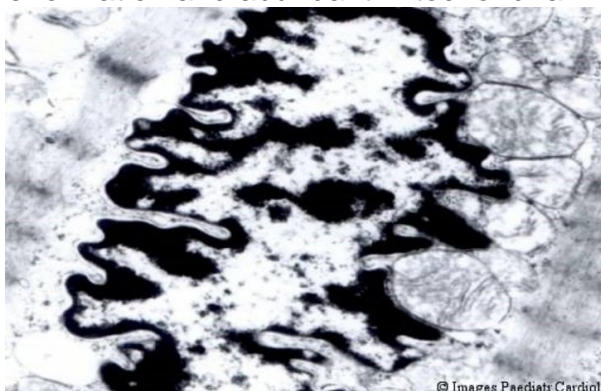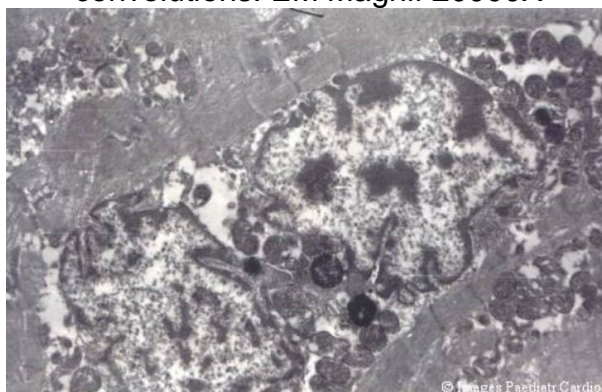

Figure 25 Electron micrograph TOF RV muscle. Convoluted nuclei with intranuclear tubule formation and abundant mitochondria. EM Magnif 30000X

Figure 26 Electron micrograph TOF RV muscle. Increased nuclear convolutions. EM Magnif 20000X

Intranuclear cytoplasmic pseudo-inclusions were seen in 3 cases and nuclear irregularities in 5 (figures 27,28).

In summary, the ultra-structural changes provided evidence for the presence of hypertrophy and degeneration (figure 29).

Degenerative changes were assessed according to the criteria described by Maron et al.,[7] as mild/early, moderate and severe. Degenerative changes are tabulated in table 4.

Data analysis of the findings and the correlative parameters was carried out using SPSS software. The relevant findings have been highlighted.

Figure 27 Electron micrograph TOF RV muscle. Hypertrophied cardiomyocytes, convoluted nuclei with intranuclear tubule formation and abundant mitochondria. EM Magnif 7000X

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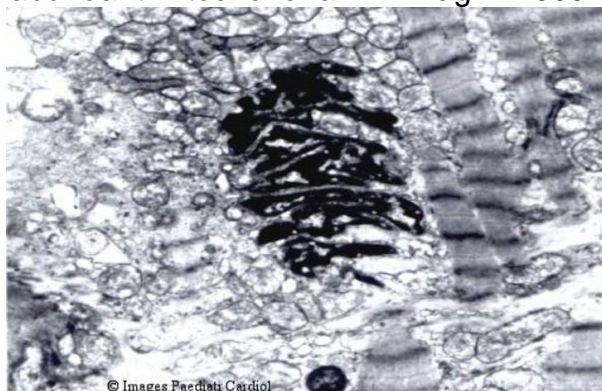
Figure 28 Electron micrograph TOF RV muscle. Convoluted nuclei with intranuclear tubule formation and abundant mitochondria. EM Magnif 20000X
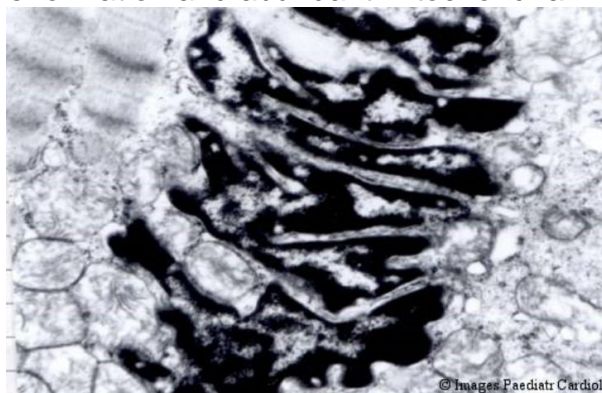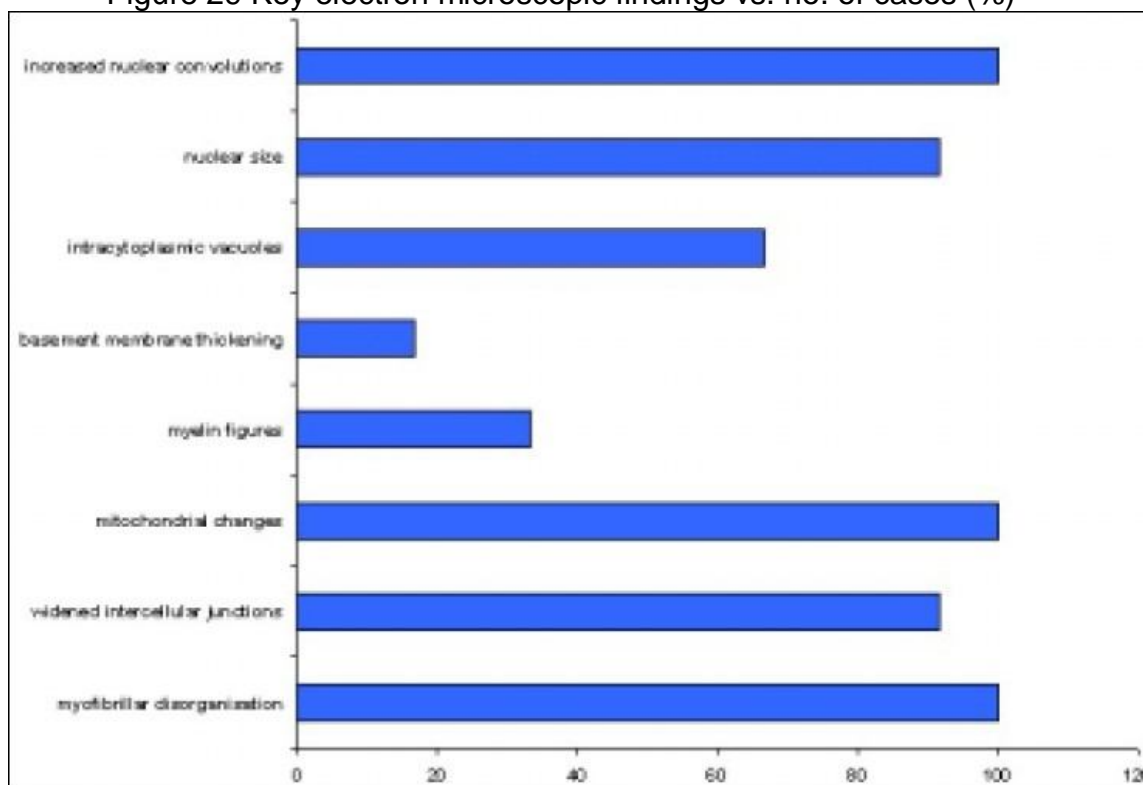

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Figure 29 Key electron microscopic findings vs. no. of cases (%)

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Table 4 Morphological changes of myocardial degeneration associated with hypertrophy

No.	Age	Myofibrillar disorganization	Myofibrillar lysis	Increase in interstitium	Loss of thick myofilaments	Intra Cytoplasmic Vacuoles	Nonparallel Myofibril	Small mito-chondria	Interstitial fibrosis	Cellular atrophy	Myelin figures	Intercellular dissociation
1.	1 yr	Mild	Mild	Slight	Mild	Nil	Few	Few	Moderate	Nil	Few	Mild
2.	2.5 yr	Mild	Focal	Mild	Nil	Few	Nil	Mild	Mild	Nil	Nil	Nil
3.	8/12	Moderate	Mild	Slight	Mild	Several	Moderate	Nil	Mild	Mild	Few	Marked
4.	2.5 yr	Mild	Mild	Slight	Mild	Nil	Few	Few	Mild	Mild	Nil	Mild
5.	2 yr	Mild	Mild	Moderate	Nil	Few	Few	Few	Mild	Mild	Nil	Mild
6.	3 yr	Moderate	Mild	Focal	Mild	Several	Moderate	Few	Mild	Moderate	Nil	Mild
7.	3 yr	Mild	Mild	Marked	Mild	Few	Few	Few	Moderate	Mild	Nil	Mild
8.	5 yr	Mild	Mild	Focal	Mild	Focal	Few	Nil	Mild	Mild	Few	Mild
9.	12 yr	Moderate	Moderate	Moderate	Mild	Several	Moderate	Few	Moderate	Moderate	Nil	Marked
10.	11 yr	Mild	Nil	Nil	Mild	Nil	Nil	Few	Moderate	Mild	Nil	Mild
11.	22 yr	Moderate	Moderate	Slight	Mild	Several	Few	Several	Mild	Moderate	Nil	Marked
12.	20 yr	Moderate	Moderate	Moderate	Mild	Few	Few	Several	Moderate	Moderate	Moderate	Moderate

Correlation of clinical/laboratory parameters with morphological changes in the myocardium

Among the laboratory investigations, hematocrit appeared to influence morphologic changes.. Based on the hematocrit, the patients were divided into 3 groups: (table 5). Group 1 included patients with hematocrit less than 45; Group II, those with hematocrit 45-60; and Group III patients had a hematocrit of more than 60.

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Table 5 Percentage of occurrence of ultra structural changes in relation to haematocrit levels

No.	Histopathologic Changes	Group I (Hct <40)	Group II (Hct 45-60)	Group III Hct>60
1.	Intercellular widening	10%	10%	60%
2.	Mitochondria	Increased in 40%	Increased in 100%	Increased and ruptured in 100%
3.	Lipid Accumulation	20%	35%	
4.	Myofibrillar disorganization	40%	30-40%	35%
5.	Nuclear convolutions	40%	40%	40%
6.	Nuclear Pseudo inclusions	80%	100%	75%
7.	Intranuclear tubules	Nil	100%	25%
8.	Increase in size & irregularity	100%	80%	50%
9.	Focal fibrosis	20%	100%	100%
10.	Perimysial Fibrosis	40%	33%	100%
11.	Perivascular fibrosis	30%	40%	50%
12.	Defective Myocytes	40%	33%	40%
13.	Sclerotic Myocytes	Nil	50%	30%
14.	Necrotic Myocytes	Nil	Nil	75%
15.	Loss of Myocytes	Nil	Nil	75%

The myocardium from group III patients (hematocrit of more than 60) showed reduction in the number of myofibrils, moderate disorganisation of myofibrils, widening of intercellular junctions, increased numbers with rupture of mitochondria and presence of necrotic myocytes. In addition, perivascular fibrosis, nuclear irregularity and increase in size as well as number of intranuclear tubules were more evident in Groups II and III. Incidentally these patients were found to have an RVEDP>6 and peak outflow gradient from 50-82 mm. Hg. Significant association was seen between haematocrit and the light microscopic finding of defective myocytes and of myocyte loss and the correlation is statistically significant (P<0.05). Higher RVEDP was associated with statistically significant loss of myocytes (p value 0.01).

Correlation of increasing age with cellular changes of hypertrophy

As mentioned earlier the mean cardiomyocyte diameter is an unequivocal determinant of hypertrophy. With increase in age, there is an increase in the micron size of the right ventricular cardiomyocytes, and the correlation co-efficient was found to be (r) = 0.5. However the correlation is not statistically significant (P= 0.14).

A careful analysis of the light and electron microscopic changes showed that apart from cardiomyocyte diameter, cellular changes of hypertrophy with increasing age were as follows: myofibrillar disorganization was of moderate degree in older patients and mild in all the young or patients, except in an 8

month old child. Intercellular dissociation was also moderate to marked in the older patients and slight in all the younger patients, except one. There was however no definite correlation of mitochondrial changes and nuclear convolutions with age. It is to be noted that with increased severity and chronicity of the disease, cellular changes of hypertrophy were modified by degenerative changes of a mild, moderate or severe nature.

Discussion

With the advent of modern safe techniques for surgical correction of Fallot's Tetralogy, myocardial function is becoming an important factor in determining the long term prognosis.[10] Morphological observations on preoperatively resected right ventricular myocardium could have a predictive value in assessing the surgical outcome in the patients, although there is a paucity of such studies in literature.[11,12,13] Effects due to hypoxia and pressure overload on the right ventricular myocardium have been found to increase with age,[1] and it has been found that the hypertrophic changes evolve into various degrees of degeneration depending on the severity and duration of the disease. The ultrastructural alterations that ensue during hypertrophy have been described at length by Maron et al.[7] In the first stage there is an increase in the energy production and protein synthesis. The second stage is characterized by a stable state of cardiac hyperfunction, which is followed by the third stage during which there is a gradual exhaustion in the heart's ability to synthesize proteins. This results in a failure to renew myofibrils and mitochondria and leads on to progressive myofibrillar damage and cellular atrophy. The same workers also analyzed the continuum of degenerative changes which occur in the late stage of hypertrophy and graded them as mild, moderate and severe. In Fallot's Tetralogy, a detailed analysis of the hypertrophic changes with or without degeneration becomes mandatory, since the severity of the right ventricular outflow tract obstruction has a significant bearing on the clinical manifestations as well as the timing for surgical intervention.[14,15]

Hypertrophy without degeneration is characterized by increase in cell size and number of myofibrils per cell. Other ultrastructural changes include focal thickening and accumulation of Z-band material, normal sarcoplasmic reticulum, focal increase in glycogen, increase in size of golgi complex and number of ribosomes and mitochondria. There is also increase in nuclear size with convolutions, but the basement membranes and interstitium are normal (figures 30,31).

S Kuruvilla, KR Balakrishnan, and U Parvathy. Right ventricular myocardium in Fallot's tetralogy: a light microscopic, morphometric and ultrastructural study. Images Paediatr Cardiol. 2004 Oct-Dec; 6(4): 1–30.

Figure 30 Electron micrograph TOF RV muscle. Cross section of hypertrophic muscle fiber and convoluted nucleus (arrow). EM magnif 3000X
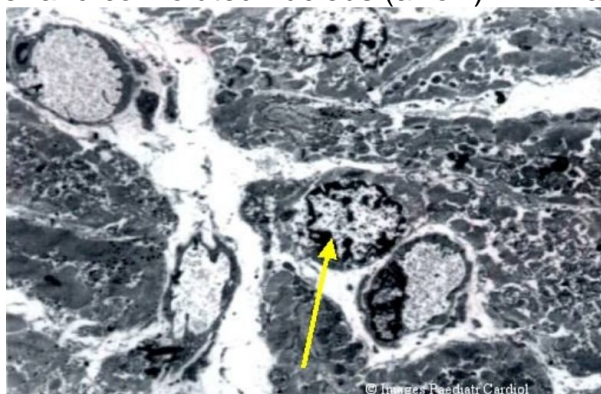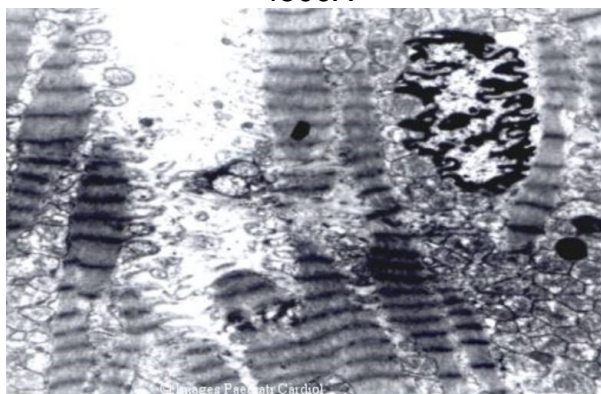

Figure 31 Electron micrograph TOF RV muscle. Hypertrophied cardiomyocytes, convoluted nuclei and abundant mitochondria. EM Magnif 4500X

Recent studies[16] show that during hypertrophy, there is a switch of the contractible proteins to the fetal and neonatal forms. The expression of alpha myosin heavy chain is replaced by that of beta myosin heavy chain, which leads to decreased myosin ATPase activity and a slower, more energetic, economical contraction.

In hypertrophy associated with mild degeneration, (figure 32) the cell size and number of myofibrils per cell are normal or increased, focal decrease in thick myofilaments, Z band abnormalities and sparse proliferation of sarcoplasmic reticulum. Changes in intracellular organelles and nuclear convolutions are the same as in hypertrophy. However there may be basement membrane thickening and increase in interstitial fibrous tissue.

S Kuruvilla, KR Balakrishnan, and U Parvathy. Right ventricular myocardium in Fallot's tetralogy: a light microscopic, morphometric and ultrastructural study. Images Paediatr Cardiol. 2004 Oct-Dec; 6(4): 1–30.

Figure 32 Electron micrograph TOF RV muscle. Longitudinal section of myocyte showing hypertrophy, mild degeneration. Note Z bands and nuclear convolutions. EM magnif 3000X

Moderate degeneration is characterized by additional changes like a marked decrease in thick myofilaments, myofibrillar disarray, abnormalities in Z bands, disorganization and extensive proliferation of the sarcoplasmic reticulum. There is partial dissociation of the intercellular junctions, decrease in size of golgi complex, number of ribosomes and a significant increase in mitochondrial size. Basement membrane is markedly thickened and there is increased interstitial fibrosis (figures 33,34).

Figure 33 Longitudinal section TOF RV myocardium showing myofibrillar disarray, nuclear irregularities and interstitial fibrosis. Semithin section Toluidine blue. LM X200
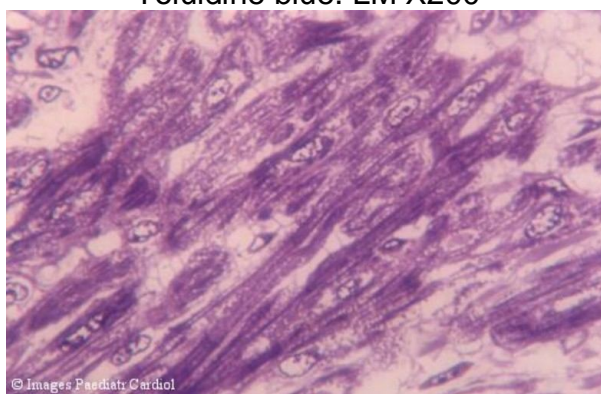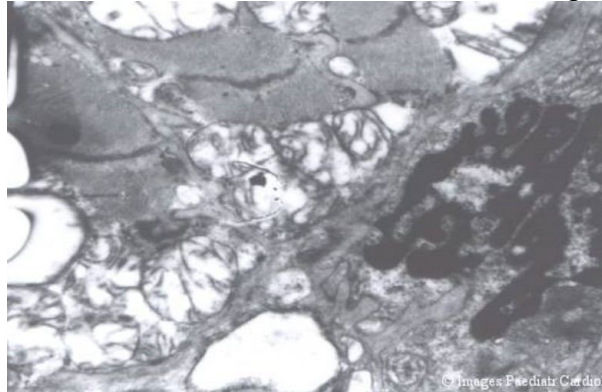
S Kuruvilla, KR Balakrishnan, and U Parvathy. Right ventricular myocardium in Fallot's tetralogy: a light microscopic, morphometric and ultrastructural study. Images Paediatr Cardiol. 2004 Oct-Dec; 6(4): 1–30.

Figure 34 Electron micrograph TOF RV muscle. Moderate degeneration-abnormal Z bands, nuclear convolutions. EM magnif 20000X

Cardiac cells showing severe, irreversible degenerative changes, by light microscopy appear atrophic and pale in toluidine blue stained semithin sections (figure 35).

Ultrastructurally, there is marked loss of contractile elements with a spectrum of changes affecting every organelle. There is marked atrophy of the cardiomyocytes, vacuolation, disorganization of myofilaments (figures 36,37) and decreased myofibrils with focal proliferation of sarcoplasmic reticulum and extensive loss of T tubules.

Figure 35 TOF RV myocardium showing irreversible degeneration seen as marked interstitial and perimysial fibrosis. The cardiomyocytes appear atrophic. Semithin section Toluidine blue LM X200
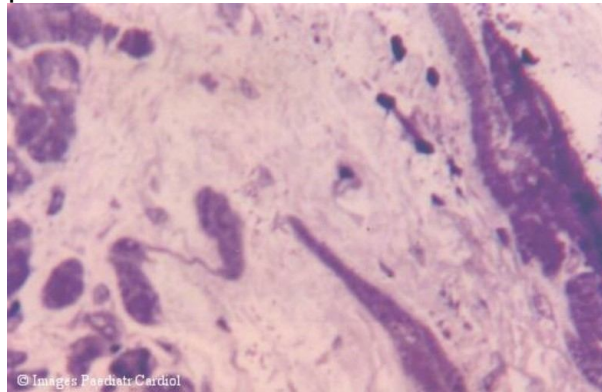
Figure 36 Electron micrograph TOF RV myocardium showing severe degeneration. The cardiomyocytes are atrophic with vacuolation and mitochondrial abnormalities. EM magnif: 7000X
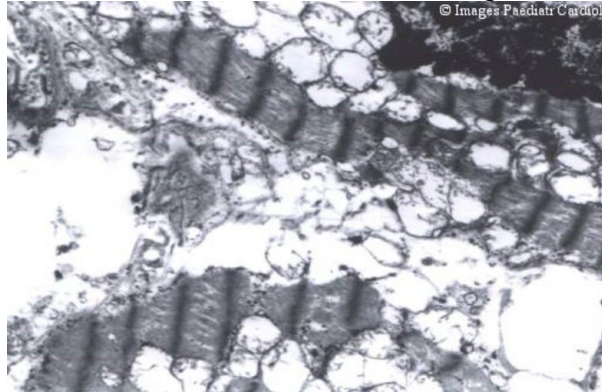

Figure 37 Electron micrograph TOF RV myocardium showing severe degeneration. The cardiomyocytes are markedly atrophic.EM magnif: 2000x
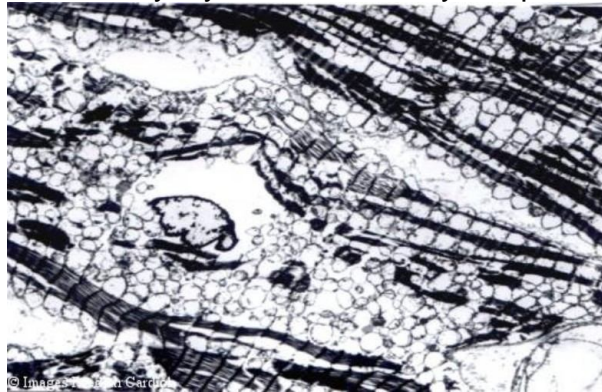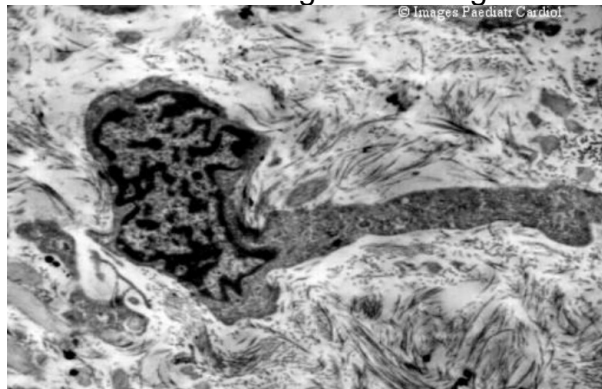

Figure 38 Electron micrograph TOF RV muscle. Interstitial fibrosis, fibroblast with nucleus and collagen. EM magnif. 7000X

Myelin figures are prominently seen and there is marked dissociation of intercellular junctions. Mitochondria of various sizes with or without damaged mitochondria may be present. Basement membrane thickening and a marked increase in interstitial fibrous tissue (figure 38) is also a prominent feature in severe degeneration.

In our analysis, the majority of patients had light microscopic and ultrastructural changes of hypertrophy associated with mild to moderate degeneration. Severe degeneration was noted in the adult patients and 3 of the paediatric age group. The clinical and haemodynamic parameters in these patients revealed a very low SPO2, presence of cyanotic spells, elevated RVEDP and haematocrit > 50. The significant morphological changes in these patients were presence of cellular atrophy, myofibrillar disorganization, myelin figures, intercellular dissociation, basement membrane thickening and marked interstitial fibrosis. The surgical outcome in these patients may be less favourable than those who had only mild to moderate degenerative changes associated with hypertrophy.

The earliest study of myocardial changes in congenital heart disease was by Vtiurin et al,[17] who noted myofibrillar edema and mitochondrial changes and correlated the severity of these changes with age. Kato et al[18] analysed the right ventricular myocardium in tetralogy of Fallot by light microscopy and morphometry. He found a significant correlation between the diameter of the cardiac myocyte with Hb levels and the age of the patients. The histopathological alterations however did not correlate with Hb, Sa O2 and PA/Ao ratio. In our study, although there was no significant increase in diameter with age, the morphological changes did show significant correlation with haemodynamic parameters, particularly Hb and SaO_2.

In a detailed ultrastructural evaluation of the crista supraventricularis in young and old patients with congenital heart disease associated with right ventricular outflow tract obstruction, Jones and Ferrans[19] observed severe degenerative changes in patients over 30 years. They attributed these changes to the stress of prolonged right ventricular hypertrophy and hypoxia and found a correlation with clinical cardiac dysfunction. In the clinical context, if total corrective surgery is performed in patients above 30 years the perioperative morbidity and late mortality rates are high. Their observations support the time-honoured concept that operative correction of congenital heart disease should be undertaken as early in life as is technically appropriate.

Tanimoto et al,[20] developed a scoring method of evaluating the light microscopic changes in right ventricular myocardial biopsies obtained pre and postoperatively in congenital heart disease. He quantified the various light microscopic parameters, such as hypertrophy, edema, vascular changes, inflammation, fibrosis and other degenerative changes. The scores were found to be significantly increased in patients as compared with controls.

A comparative assessment of pre and postoperative right ventricular wall thickness (W), diameter (D) and the ratio (W/D) in TOF patients before and after 6 months of age,[21] have shown that the right ventricular W and D decreased significantly in patients who underwent surgical repair before 6 months of age. This observation suggests that early repair of TOF has a positive effect on regression of right ventricular hypertrophy during the postoperative period. It also reduces the detrimental long-term effects of

persistent hypertrophy on myocardial function and increases the potential for occurrence of arrhythmias.

The light microscopic and ultrastructural findings in the right ventricular myocardial biopsies in TOF in the present series have been described in detail and an attempt has been made to correlate the same with the clinical and hemodynamic data of these patients. Histomorphometric analysis helped in quantitative assessment of the cell diameter, which had a bearing on hypertrophy and showed slight increase with age although the latter did not show statistical significance. This needs to be elaborately studied on a larger series of cases.

The morphological findings that were particularly prominent in this study were the increase in number of mitochondria, associated with irregularity, dilatation and rupture. These mitochondrial changes had a strong negative correlation with sPO2. Lower the sPO2; more were the mitochondrial changes (Chi 2 test 5.6000 p value 0.001, Pearson correlation - 0.68313, p value 0.014). Myocardial biopsies obtained from patients with severe right ventricular outflow tract obstruction showed marked mitochondrial alterations like irregular contours, dilatation and rupture.

Nuclear changes were also found to be fairly consistent in our series. Hypertrophic cardiomyocytes with mild to moderate degeneration showed increased nuclear convolutions. Nuclear enlargement was seen in all the cases, except one. It has been postulated,[22] that during ventricular hypertrophy, the nuclear length increases while the diameter remains normal. Also there is increased synthesis of nuclear membrane in excess of that needed to accommodate the increase in volume. This leads to increased infoldings, convolutions and other irregularities of the nuclear membrane. Presence of intranuclear tubules represent an extreme cellular response to the stimulus of hypertrophy.[23] Intranuclear tubules were observed in 5 cases (3 adults and 2 children).

Less frequently occurring ultrastructural changes include foci of myocyte damage, atrophy, intercellular dissociation and interstitial fibrosis. Some authors[24] have described cardiomyocyte damage as being defective, sclerotic and necrotic. Necrotic myocytes were seen only in patients with a haematocrit > 60% with a higher RVEDP. Statistically significant correlation existed between myocyte loss versus haematocrit. (Chi 2 test - 5.6, p. 0.05, Pearson 0.68, p value < 0.05), and between myocyte loss versus RVEDP (Chi 2 test 5.6 p value < 0.05 and Pearson 0.681, p. value 0.01).

Interstitial fibrosis which has been found to increase with worsening degeneration was noted to a greater degree in patients with raised haematocrit, RVEDP and decreased sPO2, however it was not statistically significant. Kawai et al[25] have classified myocardial fibrosis into 5 types: focal, mild and severe perimysial, perivascular and plexiform. Larger areas of myocardial disarray and severe perimysial fibrosis were seen to a greater extent in patients having hypoxic spells or with chronicity as is also our observation. Other authors[26] have found that there was marked interstitial fibrosis in patients with Fallot's tetrad who are > 5 years of age, indicating that if early repair is not done, irreversible fibrosis would reduce ventricular compliance, thus emphasizing the need for early corrective surgery.

Extensive pathohistologic studies in 104 cases of TOF by Kato,[18] showed that since irreversible light microscopic changes were first observed in patients

above 4 years of age, they proposed the timing for corrective surgery to be in children below 3 years of age. A study by Seliem and coworkers,[21] however have shown that there is significant regression of right ventricular hypertrophy when surgical correction is done before 6 months of age. This is also said to reduce the detrimental effect of longstanding hypertrophy and prevents progressive degeneration. Both these workers however did not study the ultrastructure of the right ventricular myocardium.

Summary and conclusions

In summary, therefore, from a morphological viewpoint, the most consistent and striking findings of hypertrophy with degeneration were the mitochondrial and nuclear alterations. Severe degeneration was also associated with increased amounts of interstitial fibrosis, myofibrillar lysis and disorganization and presence of myelin figures. Peripheral arterial oxygen desaturation and raised haematocrit correlated with greater mitochondrial damage. Increased nuclear convolutions were seen in patients with raised right ventricular end diastolic volume. Patients having cyanotic spells and raised haematocrit > 60% showed advanced changes viz. dilated and ruptured mitochondria, perivascular fibrosis and necrosis of myocytes.

This study was undertaken to assess the preoperative morphological changes in TOF and correlate the clinical and haemodynamic data obtained from these patients, with the degree of hypertrophy and degeneration of the right ventricular myocardium. These morphological data did have a bearing on deteriorating cardiac function particularly when changes due to severe degeneration sets in, which leads to an irreversible situation. The resulting decrease in myocardial compliance can adversely affect preoperative morbidity[27] Although these irreversible changes are expected in older patients of TOF,[1] as a result of chronicity of the disease process, we have observed these changes in 3 of our younger patients where the severity of the disease was also reflected in the hemodynamic parameters (viz lowered oxygen saturation, cyanotic spells, raised right ventricular end diastolic volume and elevated hematocrit levels).

This work provides preliminary data that the RV myocardial changes in Fallot's Tetralogy may help clinicians in determining an optimal time for surgical repair and predicting the clinical outcome. A prospective study is under way in this center on a larger number of a variety of congenital cardiac diseases, including Tetralogy of Fallot, in order to study further the spectrum of ultrastructural changes in relation to the clinical cardiac dysfunction.

Acknowledgments

References

1. Jones M, Ferrans VJ. Myocardial degeneration in congenital heart disease: A comparison of morphological findings in young and old patients with congenital heart disease associated with muscular obstruction to right ventricular outflow. Am J Cardiol. 1977;39:1051.[PubMed: 141201]

2. Hibbs RG, Ferrans VJ, Black WC, Weilbaecher DG, Walsh JJ, Burch GE. Alcoholic cardiomyopathy: An electron microscopic study. Am. Heart J. 1965;766:779.

3. Van Noorden S, Olsen EG, Pearse AG. Hypertrophic obstructive cardiomyopathy: A histological, histochemical and ultrastructural study of biopsy material. Cardiovasc Res. 1971;5:118–131.[PubMed: 5544950]

4. Nishikawa T. Ultrastructural features of the myocardium of children with dilated cardiomyopathy. Heart Vessels. 1999;14:52–56.[PubMed: 10543313]

5. Sakashita I, Matsukawa J, Ando T, Asano K. Morphological comparison of infundibular pulmonary stenosis and Tetralogy of Fallot. Jpn Heart J. 1971;12:205.[PubMed: 5315193]

6. Bojers AJJC, Van der Hearse A, Vliegen HW, Quaegebear JM, Holland I, Egas JM, Cornelisse CJ, Rohmer J, Huysmans HA. Assessment of hypertrophy in myocardial biopsies taken during correction of congenital heart disease. Thorac Cardiovasc Surg. 1988;36:137–140.

7. Maron BJ, Ferrans VS, Roberts WC. Ultrastructural features of degenerated cardiac muscle cells in patients with cardiac hypertrophy. Am J.Pathol. 1975;387:434.

8. Chalkley HW, Cornfield J, Park H. A method of estimating volume - surface ratio. Science. 1949;110:295–297.[PubMed: 17838566]

9. Arai S, Machide A, Nakamura T. Myocardial structure and vascularisation of hypertrophied hearts. Tohoku J Exp Med. 1968;95:35–54.[PubMed: 4235503]

10. Jarmakani JM, Nakazawa M, Jones I. Right ventricular function in children with Tetralogy of Fallot, before and after aortic pulmonary shunt. Circulation. 1976;53:555–561.[PubMed: 1248088]

11. Mitsuno M, Nakano S, Shimazaki Y, Taniguchi K, Kawamoto T, Kobayashi J, Matsuda H, Kawashima Y. Fate of right ventricular hypertrophy in Tetralogy of Fallot after corrective surgery. Am.J.Cardiol. 1993;72:694–698.[PubMed: 8249847]

12. Kato M, Kawashima Y, Fujita T, Mori T, Manabe M. Right Ventricular hypertrophy in Tetralogy of Fallot. Recent Adv Study Card Struc Metab. 1976;12:149–155.

13. Lee YS, Chen YC. Alteration in ultrastructure and anionic sites in basement membranes of myocardial cells and capillaries in patients with congenital heart disease due to Tetralogy of Fallot. Jpn Heart J. 1987;28:333–347.[PubMed: 3626020]

14. Rees GM, Staar A. Total correction of Fallot's Tetralogy in patients aged less than one year. Brit Heart J. 1973;35:898–901. [PMCID: PMC458726][PubMed: 4741917]

15. Pacifica AD, Bargeroi LM, Kirklin JW. Primary total correction of Tetralogy of Fallot in children less than four years of age. Circulation. 1973;48:1085–1091.[PubMed: 4751952]

16. Hunter JT, Chien KR. Signalling pathways for cardiac hypertrophy and failure. New England Journal Med. 1999;34:1276–1285.[PubMed: 10528039]

17. Vtiurin BV, Kharmas SSSH, Klamanova ES. Changes in the ultrastructure of muscle cells of the heart in congenital defects. J Kardiologia. 1963;9:26.

18. Kato M. Right ventricular hypertrophy in Tetralogy of Fallot; pathohistologic study. Jpn Assoc Thorac Surg. 1976;25:1436–1445.

19. Jones M, Ferrans VJ, Morrow A, Robert WC. Ultrastructure of crista ventricularis muscle in patients with congenital heart disease associated with right ventricular outflow tract obstruction. Circulation. 1975;51:39–67.[PubMed: 122789]

20. Tanimoto T. Histologic findings of biopsies right ventricular myocardia in congenital heart disease before and after heart surgery. Med J Hiroshima Univ. 1982;30:605–609.

21. Seliem MA, Wu YT, Glenwright K. Relation between age at surgery and regression of right ventricular hypertrophy in Tetralogy of Fallot. Pediatr Cardiol. 1995;16:53–58.[PubMed: 7784235]

22. Tounssiant M, Pfister A, Da Lage C. The nucleus of the cardiac myocyte in ventricular hypertrophy. Ultrastructural Study Bull Assoc Anat. 1984;68:59–68.

23. Ferrans VJ, Jones M, Maron BJ, Roberts WC. The nuclear membranes in hypertrophied human cardiac muscle cells. Am J Pathol. 1975;78:427–60. [PMCID: PMC1912557][PubMed: 164122]

24. Lee YS, Suug YT, Chang CH. The surface morphology of infundibular muscle inpatients with Tetralogy of Fallot. Jpn. Heart J. 1981;22:779–787.[PubMed: 7321203]

25. Kawai S, Okada R, Kitamura K, Suzuki A, Saito A. A morphometrical study of myocardial disarray associated with right ventricular outflow tract obstruction. Jpn Circ J. 1984;48:445–456.[PubMed: 6539397]

26. Hegarty A, Anderson RH, Deanfed TI. Myocardial fibrosis in Tetralogy of Fallot; effect of surgery or part of natural history? Br Heart J. 1988;59:123.

27. Maron BJ, Ferrans VJ, Jones M. The spectrum of degenerative changes in hypertrophied cardiac muscle cells: an ultrastructural study. Recent Adv. Stud. Card. Metab. 1975;8:447–466.