

ULTRASTRUCTURAL CHANGES OF LEFT ATRIAL ENDOMYOCARDIUM IN PATIENTS WITH MITRAL PARAPROSTHETIC REGURGITATION

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Paravalvular regurgitation may results in hemolysis and myocardial necrosis. The purpose of this study is to identify the presence of left atrial (LA) free wall myocardial necrosis in patients with severe paraprosthetic regurgitation (MPR) and its contribution to LDH levels.

We studied 15 patients (8 female and 7 male, the mean age 44 ± 14 years) with severe MPR who had undergone mitral valve replacement and required re-operation (range 4 to 10, mean 7 ± 3 years). All of the patients were examined by transesophageal echocardiography (TEE). Myocardial tissue specimens were taken from the left atrial wall during reoperation. Grids randomly taken were observed under the transmission electronmicroscope. Total LDH and its isoenzymes along with haptoglobin and reticulocyte count were measured. None of the patients had clinical hemolytic anemia (HA).

Electronmicroscopic examination revealed widespread necrosis of myocytes and endothelial degeneration. Ultrastructural examination demonstrated severe degeneration of mitochondria with disorganized myofilaments and interstitial edema. These findings were consistent with severe myocardial necrosis. According to the grading scale, grade 4 degeneration was determined in this series. 10 patients had elevated total LDH (> 460 IU/L) and all had high isoenzyme 1:isoenzyme 2 (LDH1/ LDH2) ratio. Electronmicroscopic findings and high LDH1/LDH2 ratio support that myocardial necrosis contributes to increase of total LDH level. We conclude that severe paraprosthetic regurgitation causing constant culprit trauma damages the myocardial tissue and results in myocardial necrosis.

Key words: Mitral paravalvular regurgitation, myocardial necrosis, lactate dehydrogenase isoenzymes, electronmicroscopy

Paraprosthetic regurgitation is a rare but serious complication of mitral valve replacement (1). Hemolysis is a well-known complication of prosthetic valve regurgitation (2-4) particularly in the presence of paraprosthetic regurgitation (5).

Hemolysis is recognized by its clinical and laboratory signs. Total lactate dehydrogenase (LDH) elevation is one of the lab indicator of hemolysis. Human sera contain several LDH enzymes and their relative proportion changes significantly in certain pathologic conditions. Isoenzyme LDH1 is present in high concentration in heart muscle, erythrocytes. In myocardial infarct LDH1 and LDH2 are increased - particularly LDH1- to yield a ratio of greater than 1. Similar LDH isoenzyme elevation occur in hemolytic anemia (HA) and also contribute total LDH elevation (6, 7).

To investigate the possible role of myocardial necrosis of left atrial (LA) free wall in patients with mitral paraprosthetic regurgitation (MPR) and its effect on total LDH and its isoenzymes, we analyzed the ultrastructural changes and lactic dehydrogenase levels obtained from 15 patients with mitral valve replacement (MVR) complicated with MPR.

MATERIAL AND METHOD

We included 15 patients (8 female and 7 male, the mean age 44 ± 14 years) complicated with severe paraprosthetic regurgitation in this study. MVR had been performed between 1989 - 1996 at Koşuyolu Heart and Research Hospital.

All replaced mechanical valves were bileaflet prostheses (St. Jude Medical valves in 10 patients and Sorin bileaflet valves in 5 patients) and implanted using separated suture technique. The sizes of the implanted mechanical valves ranged between 27 and 31mm. The mean interval between initial valve replacement and re-operation was 7 ± 3 years ranged 4 to 10 years.

None of the patients had clinical hemolytic anemia (HA). The presence of MPR was recognized and subsequently confirmed by TEE. Patients with severe paraprosthetic regurgitation underwent reoperation in the criteria of clinical decompensation.

Echocardiographic Examination

All of the patients were examined by multiplane transesophageal echocardiography (TEE) to detect the paraprosthetic regurgitation and asses its severity.

After 4 hours fasting, the patients were sedated by giving 2.5-5 mg diazepam intravenously before TEE. 10% lidocain spray was used for local anaesthesia. Echocardiographic techniques and measurements were used as recommended by American Society of Echocardiography (8). TEE was performed with a multiplane 5MHz probe (VINGMED CFM 800). Paraprosthetic regurgitation was graded semi-quantitatively according to standard criteria (9).

Mitral regurgitation was localized according to its detection at either inner or outer part of the sewing ring of the valve prosthesis. Regurgitant jets located at the outer of the sewing ring were considered paraprosthetic. The sites of the paravalvular leak were defined in the anterior portion in 3 patients, anterolateral portion in 1, posterior portion in 8 and posteromedial portion in 3 of the patients.

Biochemical Examination

The demonstration of five LDH isoenzymes was done using electrophoresis separation on agarose gels on cellulose acetate membranes. After electrophoresis separation, the reaction mixture was layered over the separation medium. The overlay and medium were incubated at 37 °C. The NADH generated over the LDH zones is detected by its fluorescence (10).

Haptoglobin (HAP) levels were tested by peroxidase activity of HAP-Hb complex separated by polyacrylamide gel electrophoresis (11).

Reticulocyte count was performed in all patients by blood smear method with Brilliant cresyl blue using the light microscope (12).

Pathological Examination

Myocardial tissue specimens taken from the site of the leak of the left atrial free wall during reoperation were fixed in 2.5% phosphate buffered gluteraldehyde solution. Postfixation was performed in 1% OsO₄.

Following embedding in Epon 812; thin sections were investigated at JEOL 1200 SX transmission electron microscope. Randomly taken grids were observed with the transmission electron microscope. An ultrastructural grading scale was used to determine myocardial tissue degeneration. These individual variables were edema, degeneration of mitochondria, disorganization of myofilaments, and abnormal appearance of Z lines. Each of the variables was evaluated as one point. Total points of each case determined the grade of degeneration.

RESULTS

Pathological examination revealed abnormal ultrastructures consistent with widespread necrosis of myocytes and endothelial degeneration.

The ultrastructural examination of the patients in the series demonstrated severe degeneration of mitochondria, disorganized myoflaments and interstitial edema. The degree myoflamentous disorganization was extremely prominent. The Z line represented abnormal accumulation of protein material. Abnormal blabbing of the endothelial cells was noticed (Figure 1-3). According to grading scale, grade 4 degeneration was determined in all of the patients.

Despite of the fact that none of the patients presented with hemolytic anemia clinically, the elevation of total LDH level (> 460 IU/L) was found in 10 patients (67%), in the remaining 5 patients (33%) total LDH level was found within normal limits (< 460 IU/L). The mean total serum LDH level of the patients (571 ± 81 IU/L) was higher than expected in all the patients. All patients had high isoenzyme 1:isoenzyme 2 (LDH1/LDH2) ratios.

DISCUSSION

Implanted prosthetic material in the heart can result in hemolytic anemia. This well-known complication was first recognized in a case of ASD repair with Teflon patch. Based on the following studies it was observed that hemolytic anemia could be the consequence of



Figure 1. Absence of myofilaments around the nuclear region (\mathcal{E}), disorganized myoflaments (\wedge) prominently damaged mitochondria (*). Interstitial edema was observed ($-$). Bar = $1 \mu\text{m}$.



Figure 2. Severe damage at the nucleus (*) empty region which is devoid of myofilaments is thought as a prominent edema (\mathcal{E}). Myoflaments are separated and they lost their regular organization (\wedge). Bar = $1 \mu\text{m}$.



Figure 3. Abnormal accumulation of protein in Z line (\mathcal{E}) results in an extreme dark appearance. Myoflaments are strictly disorganized. Degeneration of mitochondria with erased cristae (\wedge). Bar = 500 nm .

mechanical or degenerated biological prosthesis (2-4, 13).

Mild degrees of intravascular hemolysis are not uncommon patients with normally functioning prosthesis. Aortic replacement has been associated with slightly greater hemolysis than mitral replacement (14). Hemolysis has been reported to be dependent on the size of the prosthesis because significant shearing stress may develop if the valve size is small relative to the stroke volume. Other investigators have reported no significant correlation either between valve position or size and frequency of hemolysis (2, 15).

Although hemolytic anemia is a rare valve-related complication in mechanical valve replacement, this complication is a more frequently encountered clinical sign in the existence of paraprosthetic regurgitation (5). The cause of hemolytic complications has been suggested to be due to the shear stress effect of the regurgitant jet flow (16).

Hemolysis has been recognized by its clinical signs and laboratory abnormalities even without inducing anemia. Elevation of total LDH titers has been well seen during the course of hemolytic reactions. LDH1- one of the five components of LDH isoenzymes- is an essential criterion in the diagnosis of hemolysis. Especially LDH1 and LDH2 were originated from erythrocytes and cardiac tissue. LDH2 has the highest concentration of total LDH. In hemolytic process or myocardial necrosis the proportion of LDH2 over LDH1 decrease's and the LDH1 serum levels exceed LDH2 (14).

Although hemolytic anemia occurred in none of the patients included in this study, the elevation of total LDH level above normal limits (> 460 IU/L) was found in 10 patients (67%). LDH1/LDH2 ratio was greater than 1 (LDH1: LDH 2 > 1) in all of the patients.

These findings imply that the cause of the increase of LDH levels may relate to endomyocardial necrosis affecting the regurgitant jet flow. All the patients with severe MPR included in our study had biopsy proven grade 4 myocardial necrosis. The shearing stress may prominently have effect in MPR since the left ventricular contractile

forces directly strike the leak during systole and may cause endomyocardial necrosis. The incidence of myocardial necrosis was 100% in our study group.

CONCLUSION

The results of this study demonstrated that myocardial necrosis may be partially responsible for the increase in LDH isoenzyme and consequently the rise in LDH1/LDH2 ratio. Electronmicroscopic findings and high LDH1/LDH2 ratio support that myocardial necrosis is one of the main factors contributing high total LDH level. In the diagnosis of myocardial necrosis elevation of total LDH and particularly LDH1: LDH2 ratio could be used in the absence of HA. In the light of these findings we conclude that severe paraprosthetic regurgitation causing constant culprit trauma damages the myocardial tissue and results in myocardial necrosis.

Further studies of the elevations in total LDH levels are necessary in patients with complicated MPR to confirm whether the increasing LDH is related to the myocardial ultrastructural changes or hemolytic processes.

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