DEFECT IN MITOCHONDRIAL FUNCTIONS IN DAMAGED HUMAN MITRAL VALVE

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ABSTRACT

Mitochondrial diseases are a heterogeneous group of disorders in which a primary mitochondrial dysfunction is proven by morphological, biochemical, and genetic examinations. The mitral valve has important function in the regulation of blood flow from one chamber to another. Often, the mitral valve becomes abnormal with age, in Rheumatic fever or it is abnormal from birth (Congenital) or it can be destroyed by infection i.e. bacterial endocarditis and needs replacement. Myocardial function depends on energy produced by mitochondria and in any of these disease conditions, mitochondrial functions and enzyme activities may be impaired. With this in view, we analyzed the relationship between preoperative clinical conditions (as per New York heart Association) and extent of mitochondrial enzyme activities in damaged Human mitral valve in two types of heart disease such as Rheumatic Heart Disease (RHD) and Bacterial Endocarditis (BE). Thirty nine Patients undergoing cardiopulmonary bypass (CPB) for routine valvular heart surgery were included in the study. Controls include 11 normal porcine mitral valve samples without any evidence of heart disease. Mitochondrial enzymes like cytochrome oxidase (COX), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), citrate synthase (CS) and ATPase were determined. Mitochondrial COX, SDH, CS and Total ATPase activities were significantly decreased in disease condition like BE and RHD when compared with control ($P < 0.001$). On the other hand as per New York Heart Association (NYHA) preoperative clinical classification, all the mitochondrial enzymes were significantly ($p < 0.05$) impaired in class IV as compared with NYHA class I, II and III. Present study shows that impairment in the mitochondrial enzymes activities are more pronounced in bacterial endocarditis (BE). It also indicates that damage to mitochondrial enzymes are most pronounced in NYHA class IV.

KEY WORDS

Human heart, Mital valve, and Mitochondrial functions.

INTRODUCTION

The Mitral valve is the inflow valve into the left side of the heart, which allows blood to move from the left atrium into the left ventricle (1). Mitochondria play a pivotal role in cellular metabolism, especially in energy production. Myocardial function depends on ATP supplied by oxidation of several substrates. In adult heart, this energy is obtained primarily from fatty acid oxidation through oxidative phosphorylation. However, the energy source may change depending on several factors such as substrates availability, energy demands,

oxygen supply and metabolic condition of an individual (2). Myocardial recovery from ischemic injury requires a substantial generation of energy to repair damage to cellular processes and allow for return of contractile force after reperfusion. The mitochondria are the subcellular sites of this vital energy generating function, their recovery from ischemic injury is of fundamental importance. Cardiac mitochondria are complex structure and this complexity is particularly evident in the molecular structure of the crista membrane, which organizes the enzyme molecules that are responsible for phosphorylation and that ensure the efficiency of energy generation (3,4). Study of Mitochondrial function in the failing heart has previously been limited so often to experimental animals, where the findings have been conflicting.

Oxidative phosphorylation and the content of high-energy phosphate compound have been reported to be normal in

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some of these studies (5), where as in others defective energy production has been observed (6). There is not much information available on mitochondrial function in normal or diseased human mitral valve.

In the present study we examined, the relationship between preoperative clinical conditions (as per New York heart Association) and extent of mitochondrial enzyme activities in damaged Human mitral valve in two types of heart disease such as Rheumatic Heart Disease (RHD) and Bacterial Endocarditis (BE).

MATERIALS AND METHODS

Thirty nine Patients (62% male) undergoing cardiopulmonary bypass (CPB) for routine valvular heart surgery were included in the study. Controls include 11 normal porcine mitral valve samples without any evidence of heart disease. Results were performed by two ways first, on the basis of findings in clinical examination patients of mitral valve replacement were allocated to their respective class as per New York Heart Association (NYHA) functional classification (7):

- Class I** Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity doesn't cause undue fatigue, palpitation, dyspnoea, or anginal pain.
- Class II** Patients with cardiac diseases resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in undue fatigue, palpitation, dyspnoea, or anginal pain.
- Class III** Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnoea, or anginal pain
- Class IV** Patients with cardiac disease resulting in ability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present at rest. If any physical activity is undertaken, discomfort is increased.

Patients were distributed according to NYHA class: **18% of NYHA I, 46% of NYHA II, 18% of NYHA III, and 18% of NYHA IV** and second, by the types of valvular heart disease. Out of 39 patients, 36% patients of RHD and 42% patients of BE and remaining for control. L.T.M. Medical College and General Hospital, ethics committee gave approval and informed consent was obtained from each patient.

The general methods utilized for MVR were as described by Morrow and Clark (8). Mitral valve was placed in ice-cold medium containing 0.225M Mannitol, 0.075 M Sucrose, 0.05 mM EDTA (pH = 7.8), Unneutralized Tris buffer (1M) (MSE Medium)

Isolation of Mitochondria

Mitral valve are washed in cold MSE medium and finely chopped with a pair of scissors. 0.05 ml (1M) Tris-buffer is added to the tissue and mixture was transferred to 10 vol. of MSE medium. The tissue mince was transferred to a smooth walled homogenizing vessel and disrupted with a loose fitting motor driven Teflon pestle with 15 strokes. The homogenate was centrifuged at 700 x g for 10min. The supernatant was centrifuge at 10,000 x g for 10 min. to obtained mitochondrial pellet. This pellet was washed with MSE medium, recentrifuged at 10,000 x g for 10 min (9).

Enzyme assays

Mitochondrial pellet was used for the estimation of Succinate dehydrogenase (10), Malate dehydrogenase (11) & Citrate Synthase (12) activity.

Determination of Cytochrome Oxidase

The cytochrome activity was measured according to Smith (1955). 1.0 ml solution contains 10mM potassium phosphate buffer pH 7.4, 200g mitochondrial protein and 0.5 mM reduced cytochrome C. Reaction was started by addition of cytochrome c and decrease in absorbance was measured at 550 nm for 3 min (13).

ATPase

Mitochondrial suspension was incubated at 37°C for 60 min in 0.5 ml medium containing 2 mM ATP, 100 mM NaCl, 20 mM KCL, 5 mM MgCl₂, 1mM EDTA in 50 mM Tris-HCl (pH =7.0). The tubes were chilled immediately and centrifuged at 200 x g for 10 min. *Inorganic phosphate* liberated in the supernatant was estimated according to Fiske & Subbarow method (14). *Protein Estimation* was carried out according to Lowry et al with crystalline bovine serum albumin as standard (15).

Statistical Analysis

Statistical comparison of data was done using Student's t-test and NYHA class I, II, III, IV were compared with each other by using One-Way ANOVA. Values were expressed as

means $\pm$ standard deviation (SD). Sigma stat version 3.0 was used for statistical analysis. *P*-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Demographic data showed average age of 30 ± 9.87 years with weight 49 ± 11.2 kg. Maximum patient (46%) belonged to NYHA class II and 42% patients belonged to Bacterial endocarditis.

Mitochondrial cytochrome oxidase activity in heart diseases are shown in Table 1. Cytochrome oxidase activity is significantly decreased in BE and RHD as compared with control ($P < 0.001$).

Mitochondrial ATPase activities in heart diseases are shown in Table 2. Total-ATPase activity was decreased by 50% in RHD and 27.27% in BE when compared with control.

Activities of mitochondrial Kreb's cycle enzymes like Citrate Synthase (CS), succinate dehydrogenase (SDH) and Malate dehydrogenase (MDH) are shown in Table 3. CS and SDH activity showed significant decrease in disease condition like RHD and BE when compared with control ($P < 0.001$). MDH activity was decreased to about 41.46% in RHD and 39.02% in BE, as compared with control ($P < 0.05$).

Mitochondrial Enzymes activities in terms of preoperative clinical condition as per New York Heart Association (NYHA) are shown in Fig.1. All the enzyme activities were significantly decreased in NYHA class IV in comparison with NYHA Class I, II and III. Maximum decrease was observed in cytochrome oxidase activity in all four NYHA classes as compared with other enzymes. Kreb's cycle enzymes like SDH, MDH, CS and ATPase activity were significantly decreased in class IV

Values are given % decrease over the control. All values are compared as per NYHA classification. Number of Observation: NYHA I = 7, NYHA II = 18, NYHA III = 7, NYHA IV = 7.

Table 1
Mitochondria Cytochrome oxidase Activity in heart diseases

Sample	Cytochrome Oxidase nmole of Cyt. reduced/mg protein	% of control
Control (11)	0.66 ± 0.01	100
BE (20)	0.032 ± 0.01 *	4.85
RHD (19)	0.054 ± 0.01 *	8.18

* $p < 0.001$ Compared with values for Control.

Table 2
Mitochondrial Total ATPase activity in Heart Diseases

Sample	Total - ATPase μ mole of Pi/min/mg protein	% of Control
Control (11)	0.44 ± 0.13	100
BE (20)	0.12 ± 0.02 *	27.2
RHD (19)	0.22 ± 0.07 °	50.0

* $p < 0.001$ Compared with values for Control. ° $p < 0.05$ compared with values for control.

Table 3
Mitochondrial Kreb's Cycle Enzymes activity in Heart Disease

Sample	Citrate Synthase	Succinate Dehydrogenase	Malate Dehydrogenase
	μ mol of CoA/mg Protein	μ mol DCIP reduced/min/mg protein	U/mg protein
Control (11)	11.7 ± 4.3	9.11 ± 2.5	0.041 ± 0.009
BE (20)	4.62 ± 2.9 * (39.48)	0.84 ± 0.20 * (9.22)	0.016 ± 0.004 # (39.02)
RHD (19)	4.89 ± 1.9 * (41.79)	2.8 ± 0.14 * (30.73)	0.017 ± 0.009 # (41.46)

* $p < 0.001$ Compared with values for Control. # $p < 0.05$ compared with values for control; Parenthesis indicates % of Control.

when compared with other three classes ($P < 0.05$). Under these conditions, mitochondrial protein contents did not show any significant change.

DISCUSSION

This is first case report study of mitochondrial function in disease human mitral valve. The mitral valve is the most common sites of valvular heart disease, because of their location on the left side of the heart. The left chambers have a greater workload because they pump blood to the entire body, whereas the right chambers push blood only to the lungs. Any abnormalities in the valves of the left atrium and

ventricle are more likely to produce symptoms (16). All valves have thin flaps of muscle tissue, called leaflets or cusps that open to let the blood through and close to prevent it from flowing backward. The leaflet portion is mostly affected in infection (17). Patients infected valves are removed by Valve replacement surgery. Mitral valve replacement (MVR) was done by cardiopulmonary bypass (CPB). CPB is widely used to maintain systemic perfusion and oxygenation during open-heart surgery. Tissue perfusion & oxygenation during CPB is achieved by adjusting flow rate, temperature, gas flow, and hemoglobin to maintain O₂ delivery. Tissue hypoperfusion may occur due to low flow CPB hypothermia extreme hemoglobin and excessive neurohormonal activation. This leads to an anaerobic condition in which oxidative phosphorylation is not possible and ATP is produced from pyruvate (18). Few studies have shown that patient's complications and sequel heart valve disease such as breathlessness is a major symptom of heart valve disease, along with swelling of the ankles. Others symptoms may include fatigue, fainting, palpitations and chest pain (19, 20). Our patients have shown similar symptoms as given as above.

Studies have been carried out with mitochondria isolated from damaged human heart mitral valve to determine whether there was an abnormality in the functional changes of these mitochondria. Mitochondrial cytochrome oxidase was highly affected in RHD and BE. In NYHA functional classification, COX in stage IV is significantly affected. COX by NYHA functional class is decreased to 6.21% in NYHA I, 5.60% is in NYHA II, 4.24% in NYHA III and 3.03% is decreased to NYHA IV. Maliuk (20) showed that significantly decreased in mitochondrial COX activity in patients with mitral valve insufficiency. Viti *et al* (21) have reported a decrease in cytochrome in mitochondria in patients undergoing mitral valve insufficiency. Mitochondria play a very important role in kreb's cycle enzymes.

All enzymes of kreb's cycle studied are drastically decreased in all the disease condition. Similar to our observation, a decreased in Citrate synthase, succinate dehydrogenase and Malate dehydrogenase enzyme activity in mitochondria has been noted by Drahota *et al* (22). CS is the best marker enzyme for mitochondria and more specifically, the matrix fraction. NYHA functional classification shows that Stage IV is highly affected, where SDH, MDH and CS were decreased by 91.1%, 65.8% and 61.8% respectively, when compared with control. ATPase activity was drastically impaired in bacterial endocarditis (BE) diseases (27.2%; % of control) *P* values are highly significant in BE (*P*<0.001). It is also seen

that ATPase activity was highly affected in stage IV patients. Heart work depends on ATP and is inhibited by oligomycin, which causes rapid arrest with decrease of ATP. Recently it has been found that it is possible to obtain single beating heart cells from embryonic chicken hearts and young rat hearts which continue to beat rhythmically for upto 4 days (23). They form synchronously-beating nests of cells, the beating of which depend on ATP and are arrested by oligomycin. Beating heart cell must have a mechanism for maintaining a high steady state of ATP, perhaps involving creatine phosphate (24). Cytochrome c activity was decreased in heart muscle mitochondria have been reported by Viti *et al* (21). Many studies in the past 10 years have demonstrated that the production of ATP by heart mitochondria is impaired in heart failure (6, 25-26).

There are 2 important aspects in considering possible involvement of mitochondria in heart valve damage. These are 1) Severity of failure and 2) Type of failure. Relationship between mitochondrial damage & pathogenesis of heart valve damage can be either cause based or effect based, with damaged mitochondrial function leading to the heart valve defect; or the reduction in mitochondrial function being an effect of valve defect.

CONCLUSION

Present study indicates impaired mitochondrial functions in BE & RHD. Further the impairment was more in NYHA class IV when subjects were classified according to the clinical condition specified as NYHA protocol.

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