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Original Article

Diagnostic value of endomyocardial biopsy for endomyocardial fibrosis

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Abstract: Endomyocardial fibrosis (EMF) is a restrictive cardiomyopathy of unknown etiology. It is mainly prevalent in the tropical and subtropical countries. The aim of this study was to evaluate the diagnostic value of endomyocardial biopsy (EMB) for EMF, and detect the relationship between Coxsackie virus B_3 (CVB_3) and EMF in the Chinese patients. The right ventricular EMB was performed in 18 patients who had been diagnosed as EMF by angiocardiography, echocardiography and/or magnetic resonance imaging. CVB_3 in eight patients' endomyocardial samples was detected with CVB_3 cDNA probe by *in situ* hybridization method. The successful rate of EMB was 83.3% (15/18 cases) in this study. The major histopathological findings of EMB were endocardial thickened in 11 cases (73.3%), endocardial collagen fibration in 14 cases (93.3%), endocardial hyalinization in 7 cases (46.7%), small vessel growing in the endocardial deep layer in 10 cases (66.7%), wispy fibrotic tissues extend into subendocardial myocardium in 7 cases (46.7%), myocardial interstitial fibrosis in 7 cases (46.7%), myocardial hypertrophy, degeneration or amyotrophy in 9 cases (60.0%), and inflammatory cell (lymphocytes and macrophages) infiltration in 7 cases (46.7%). The histopathological findings were found to be diagnostic in 12 patients (80.0%). No positive hybridization signal was detected in all of the eight patients. The results of the present study suggest that EMB is a quite useful diagnostic technique for EMF, but the pathogenesis of EMF may be not related with CVB_3 direct infection.

Keywords: Endomyocardial fibrosis, endomyocardial biopsy, Coxsackie virus B_3, in situ hybridization, histopathological diagnosis

Introduction

Endomyocardial fibrosis (EMF) is also known as Davies' disease after the detailed descriptions given by Davies in 1948 [1]. It occurs almost exclusively in tropical and subtropical countries, especially in Uganda, Nigeria, Ivory Coast, Brazil and India [2]. According to the WHO/ISFC Task Force recommendation, the disease has been included in the category of restrictive cardiomyopathy. The essential features of EMF are the formation of fibrous tissue on the endocardium and to a lesser extent in the myocardium of the inflow tract and apex of one or both ventricles. It results in endocardial rigidity, atrioventricular valve incompetence secondary to papillary muscle involvement, and progressive reduction of the cavity of the involved ventricle leading to restriction in filling and atrial enlargement. Superimposed thrombosis and calcification are common at advanced stages of the disease. The first Chinese case of EMF was described in 1965 [3]. Although the prevalence of EMF in China is unknown, EMF accounted for 3% of the hospitalized patients with primary cardiomyopathy in the First Affiliated Hospital of Guangxi Medical University [4]. The diagnosis of EMF and its differential diagnosis from other types of restrictive heart disease, especially constrictive pericarditis, may often be difficult in areas where the EMF is not common because of the lack of specific clinical manifestations. In addition, group B Coxsackie viruses are considered to be the most common cause of viral myocarditis in both humans and animals. Coxsackie viruses not only cause myocarditis but may also be responsible for dilated cardiomyopathy [5]. However, it is unclear whether these viruses are also associated with the pathogenesis of EMF. Thus, this study was undertaken to evaluate the diagnostic value of endomyocardial biopsy (EMB) for EMF, and

Table 1. Clincal data and histopathological findings of EMB (A to G) in 18 EMF patients

SerialNo.	Age/Sex	UCG	ACG	MRI	ECT	A(μm)	B	C	D	E	F	G	HPD
1	15/F	+	+	..	..	100	+	-	+	+	+	+	+
2	40/M	±	+	..	..	250	+	+	+	+	+	+	+
3	43/F	±	+	..	..	55	+	+	+	+	+	-	+
4	24/M	-	+	..	+	?	+	+	-	-	-	-	+
5	39/F	-	+	..	+	?	+	-	-	-	-	-	-
6	50/M	-	+	..	..	150	+	-	+	+	-	-	+
7	39/F	-	+	..	..	60	-	-	-	-	-	+	-
8	55/F	-	+	..	..	100	+	-	+	+	+	+	+
9	35/F	+	+	..	..	80	+	-	+	-	+	+	+
10	20/M	-	+	..	..	100	+	-	+	+	+	-	+
11	52/M	-	+	+	..	*	..	..	..	..	..	..	..
12	52/M	-	+	..	..	80	+	+	+	-	+	+	+
13	17/F	-	+	..	+	?	+	-	-	-	-	-	-
14	22/M	±	+	..	..	*	..	..	..	..	..	..	..
15	30/F	±	+	..	..	?	+	+	-	-	-	-	+
16	40/F	-	+	..	..	*	..	..	..	..	..	..	..
17	46/M	+	+	+	..	120	+	+	+	-	+	+	+
18	19/F	+	+	+	..	100	+	+	+	+	+	-	+

UCG, echocardiography; ACG, angiocardiography; MRI, magnetic resonance imaging; ECT, endocardiectomy; EMB, endomyocardial biopsy; A. endocardium (μm); B. endocardial collagen fibration; C. endocardial hyalinization; D. small vessel growing in the endocardial deep layer; E. wispy fibrotic tissues extend into subendocardial myocardium; F. myocardial hypertrophy, degeneration or amyotrophy; G. inflammatory cell infiltration; HPD, histopathological diagnosis; +, positive; -, negative; ±, suspicious; ?, the EMB sample does not contain endocardium or endocardium was not seen; *, EMB failed (available specimen was not obtained); .., the examination was not performed.

detect the relationship between Coxsackie virus B_3 (CVB_3) and EMF in the Chinese cases.

Methods and materials

Patients

The patients in this study were divided into EMB group and pathological examination (autopsy and endocardiectomy) group. There were 18 patients (8 males, 44% and 10 females, 56%) in EMB group, aged from 15 to 55 years, with a mean age of 35.44 ± 13.25 years. The predominant clinical picture was an insidious and progressive heart failure. Other symptoms included cough, weakness, palpitations, abdominal swelling, edema, arrhythmia, and dyspnea. The disease course was from 1 to 17 years, with an average of about 3 years. There were 7 cases (4 for autopsy and 3 for endocardiectomy) in pathological examination group, aged from 16 to 64 years, with a mean age of 32.40 ± 17.85 years. The diagnosis of EMF was based on the clinical manifestations and the findings of hemodynamics, echocardiography, angiocardiography and cardiac magnetic resonance imaging or autopsy (**Table 1**). None of the patients had peripheral eosinophilia. This study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University, and conducted according to the Declaration of Helsinki. Written informed consent was obtained from each subject prior to the procedure of EMB.

Right ventricular EMB

Transfemoral right ventricular EMB was performed in all patients. Following local anaesthesia of 2% lidocaine 5 ml, a 7F sheath is introduced into the right femoral vein. The EMB of the ventricular septum was performed from the right ventricular side. At least one to five biopsy specimens were taken with a Cordis bioptome advanced through the 7F sheath to reach the right ventricle. The biopsy specimens were fixed in 4% buffered formaldehyde. After conventional processing, 4 μm thick sections were cut and stained with hematoxylin-eosin, Masson's trichrome and Verhoffs Van Gieson

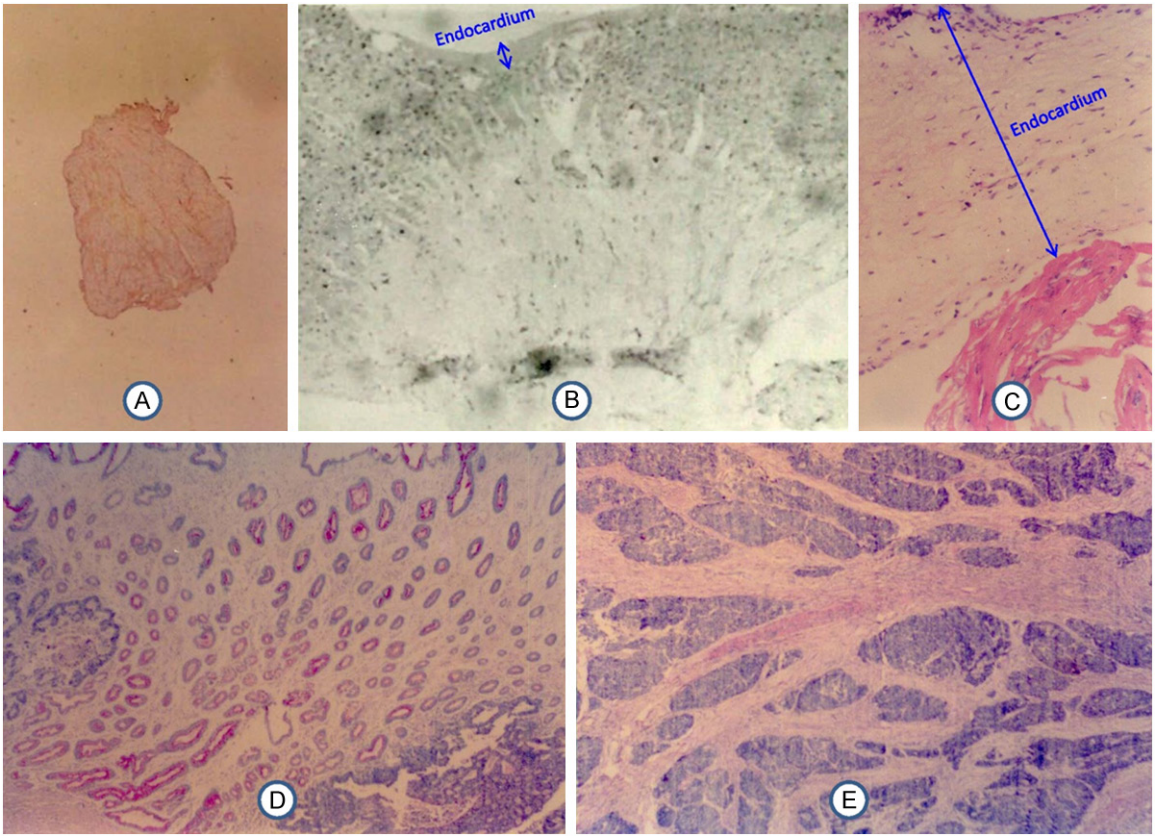

Figure 1. Histopathological findings of EMF in EMB. A. EMB specimen, Hematoxylin × 40. B. Endocardial thickening ≥ 50 μm, HE × 100. C. Endocardial thickening ≥ 50 μm, HE × 200. D. Many small blood vessels in the endocardial deep layer, HE × 400. E. Wispy fibrotic tissues extend into subendocardial myocardium, HE × 100.

stain. The sections were examined in detail for changes in the endocardium, myocardium and interstitium.

In situ hybridization

EMB specimens were fixed in fixative solution (1.5% paraformaldehyde/1.5% glutaraldehyde/0.1 M sodium phosphate buffer, pH 7.2) for 2 h at 4°C and embedded in paraffin. Serial sections (4 μm) were mounted on microscopic slides that had been coated with 3-aminopropyl-3-ethoxysilane. Before hybridization, the slides were pretreated as follows: 20 min in 0.2 M HCl at room temperature, 30 min in 2 × SSC at 70°C, and 15 min in 20 mM Tris·HCl, pH 7.4/2 mM $CaC1_2$/proteinase K (1 μg/ml) at 37°C [6]. Slides were washed twice in distilled water and then dehydrated in graded ethanol solutions (70% and 95%). Hybridization mixture (14 μl: ^{3}H-labeled CVB_3 cDNA probe 200 ng/ml, heated to 100°C for 5 min, 10 mM Tris·HCl pH 7.4, 50% deionized formamide, 600 mM NaCl, 1 mM EDTA, 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.05% bovine serum albumin, 10% dextran sulfate, 10 mM dithiothreitol, sonicated denatured calf thymus DNA 200 μg/ml, myocardial total RNA 1 mg/ml, and rabbit liver tRNA 200 μg/ml) was applied to each slide, siliconized coverslips were mounted and sealed with rubber cement, and hybridization was allowed to proceed at 25°C for 48 h. Slides were then washed for 18 h at 37°C in 10 mM Tris·HCl, pH 7.4/2 × SSC/50% formamide/1 mM EDTA followed by 1 h at 55°C in 2 × SSC, then rinsed in 2 × SSC, and dehydrated in graded ethanol solutions containing 300 mM ammonium acetate. Hybridized preparations were autoradiographed with NTB2 nuclear track emulsion (Eastman) diluted 1:1 with 600 mM ammonium acetate [7, 8]. The positive control sections were the rat myocardial slices which have been confirmed as CVB_3 myocarditis.

Histopathological diagnosis criteria

The histopathological diagnosis criteria were as follows: (1) Endocardial thickening and hyaline

degeneration, ≥ 50 μm; (2) Thickened endocardium was mainly collagen fibers, without elastic fibers or few local elastic fiber hyperplasia; (3) Wispy fibrotic tissues extend into subendocardial myocardium; (4) There were many small blood vessels in the endocardial deep layer [9, 11].

Statistical analysis

The statistical analysis was carried out using the statistical software package SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Endocardial thickness in the two study groups was expressed as mean ± standard deviation, and was tested by the Student's unpaired *t*-test. Qualitative variable was expressed as percentage and tested by the chi-square test. A *P* value of less than 0.05 was considered statistically significant.

Results

EMB and histopathological findings

The operation of EMB was completed and obtained at least one ideal specimen in 15 patients. The procedure was repeated more than 10 times but not obtained available specimen in 3 patients. Thus, the successful rate (at least one available specimen was obtained) of EMB was 83.3% (15/18 cases) in this study. There were no serious complications. The major histopathological findings were endocardial thickened in 11 cases (73.3%), endocardial collagen fibration in 14 cases (93.3%), endocardial hyalinization in 7 cases (46.7%), small vessel growing in the endocardial deep layer in 10 cases (66.7%), myocardial interstitial fibrosis in 7 cases (46.7%), myocardial hypertrophy, degeneration or amyotrophy in 9 cases (60.0%), and inflammatory cell (lymphocytes and macrophages) infiltration in 7 cases (46.7%). Eosinophils were not seen in the endocardium. The histopathological findings were found to be diagnostic in 12 patients (80.0%; **Table 1** and **Figure 1**). The degree of endocardial thickness was lower in EMB group than in pathological examination group (108 ± 54 *vs*. 4229 ± 2992 μm, $t = 4.876$, $P < 0.001$). The incident rate of fibrotic tissues that extend into the adjacent myocardium was also lower in EMB group than in pathological examination group (46.7% *vs*. 100.0%, $P < 0.05$). There were no differences in the remaining parameters between the EMB and pathological examination groups.

In situ hybridization results

No positive hybridization signal was detected in all of the eight specimens (EMB, n = 5; endocardiectomy, n = 2; and autopsy, n = 1; at least five sections were used for each specimen). But CVB_3 positive hybridization signals were found in the positive control sections.

Discussion

EMB is a well-accepted diagnostic method for a number of myocardial disorders and detecting graft rejection after heart transplantation, but very few EMB studies in cases of EMF are available [9, 11]. In the present study, we showed that endocardial thickening, endocardial collagen fibration, endocardial hyalinization, small vessel growing in the endocardial deep layer, and wispy fibrotic tissues that extend into the adjacent myocardium were the main histopathological changes of EMF. These main histopathological features were also the histopathological diagnosis criteria of EMB for EMF. As compared with the pathological examination (autopsy and endocardiectomy) group, we found that the degree of endocardial thickness in EMB group was significantly lower. The incident rate of fibrotic tissues that extend into the adjacent myocardium was also lower in EMB group than in pathological examination group. The reason for these discrepancies is not completely clear. One of the reasons may be that the EMB specimen is too small. It is also possible that an EMB specimen often cannot contain endocardial and myocardial tissues simultaneously. In addition, the patients who were performed EMB may be on their early changes of EMF, whereas the patients who were performed surgical therapy and/or autopsy may be on their late fibrotic stage of EMF. These findings suggest that EMB diagnostic EMF also has some limitations. According to the results of Somers et al. [11], the total success rate with single and multiple biopsies in 64 patients with cardiomyopathy (EMF, 49 patients) approached 80 per cent. Most of the failures occurred in patients with EMF where the bioptome tends to slide over the smooth fibrous endocardium. In this study, the success rate with the technique was 83.3% (15/18 cases). The procedure of EMB was repeated more than 10 times but not obtained available specimen in 3 patients. During the procedure of EMB, the following situations often took place: (1) The bioptome was

not easy to enter right ventricle due to huge right atrium, or it is easy to exit. (2) The bioptome jaws tends to slide over the smooth fibrous endocardium, or there is a sense of biting calcified tissues. (3) Rigid sense of right ventricular apex. (4) There was no arrhythmias such as premature ventricular contractions, paroxysmal ventricular tachycardia when the biting mouth of the bioptome contacted with the right ventricular wall. (5) The procedure of EMB was repeated more than 10 times but not obtained available specimen, or the EMB specimen was a mural thrombus. But if these situations happen, it is also suggested that there is a possibility of EMF. In addition, if right ventricular EMB was performed in the patients with left ventricular EMF, the result is often negative and the diagnosis is often missed [12].

The etiology of EMF is still unknown even 68 years after its description [1, 13]. Parasitic infections [14], nutritional disturbances [15], high plantain (banana) diets, anoxia, geochemical factors [16] and immunological reaction have all been considered possible causes but none is considered the likely cause [4]. A few patients have been described in families, suggesting that genetic factors may play a role in the etiology. It has already been pointed out that, many years ago, the possible association of eosinophilia in the pathogenesis of EMF in the tropics had been considered, but no relationship of EMF to eosinophilia exists in these patients [4]. It was also considered to be associated with some viral infections. Glassy et al [17] reported a case of Löffler's endocarditis (60-year-old woman) with multiple lymphadenopathies, blood eosinophilia and acute heart failure. The eosinophilia developed over a six-month period to a maximum of 54.5% eosinophiles for 7600 × 10^9 leukocytes/L. Autopsy disclosed parietal endocarditis with endomyocardial fibrosis and mural thrombi, principally in the inflow tract of the left ventricle, lesions typical of the fibroplastic parietal endocarditis described by Löffler. Examination of the lymph nodes at autopsy showed a disseminated viral infection probably due to a virus of the herpes group, as was confirmed by electron microscopy. These results indicate that EMF may be associated with some viral myocarditis. In the present study, however, we failed to detect the positive hybridization signal in all of the eight EMF patients. This finding is consistent with the results of serological examination by Ijaola et al. [18]. They investigated the relationship between EMF and the presence of antibodies against CVB_{1-6}, 16 arboviruses and Toxoplasma gondii in 8 EMF patients. The results showed that none of the eight EMF patients had antibodies against any of the CVB_{1-6} in their sera. There was no significant difference in the distribution of antibody titres to the arboviruses between the EMF patients and matched controls. But all of the eight EMF patients had high antibody titres against Toxoplasma gondii [18]. These results suggest that the pathogenesis of EMF may be not related with CVB_3 direct infection. Iglezias et al. [2] investigated the occurrence of cardiotropic infective agents directly in the endomyocardium of patients presenting EMF. Half of the patients with EMF showed infective genomes in the ventricular endomyocardium. Two patients were positive for enteroviruses (EV), two for cytomegalovirus (CMV), one for both EV and CMV (42%, 5/12), and one for Toxoplasma gondii (8%, 1/12). The RT-PCR or PCR was always repeated to confirm the initial positive amplification. Sequencing analysis of the viral CMV PCR products showed the follow homology: AF413626-human herpesvirus 5 strain 17A putative glycoprotein (UL20a) gene (identity 99.8%), X17403-human cytomegalovirus strain AD169 complete genome (identity 95.7%), and AF413627-human herpesvirus 5 strain 19A putative glycoprotein (UL20a) gene (98.3% identity). The EV PCR products showed the homology comprised between 85% and 90% for different enteroviral serotypes (i.e., coxsackievirus, echovirus, poliovirus, enterovirus).

Conclusions

The results of the present study suggest that EMB is a quite useful diagnostic and differential diagnostic tool for EMF, but the success rate of EMB procedure and obtaining sufficient EMB samples remain two main impact factors. Not surprisingly, in some patients with dense collagen scars and calcified thrombus over the ventricle it is not easily possible to obtain sufficient EMB specimens. The pathogenesis of EMF may be not related with CVB_3 direct infection.

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Disclosure of conflict of interest

None.

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