Diagnosis of Viral Myocarditis in Children with Clinical Suspicion of Myocarditis by Multiplex Real-time PCR in Birjand Vali-Asr Hospital, East of Iran

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Research article

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Abstract

Background Viral myocarditis (VMC) is one of the common heart diseases to endanger human health in different age groups, especially children. The aim of this study was diagnosis of viral myocarditis in children with clinical suspicion of myocarditis by multiplex Real-time PCR in Birjand Vali-Asr hospital. **Methods** This cross-sectional research conducted on 19 patients with clinical suspicion of myocarditis who had evidence of microbial infections in recent weeks. Routine electrocardiogram and dynamic ECG examinations, auxiliary laboratory testing and echocardiographic evaluation were performed. Multiplex Real-time PCR by Neuro kit was used for detection of viral agents in blood or pericardial fluid samples. **Results** The results indicated that 4 out of 19 (21.05%) patients with myocarditis were found to be virus positive. The spectrum of viral agents was included HHV-6/7, Parvovirus B-19, EBV, VZV and Adenoviruses. A co-infection of HHV-6/7 and Parvovirus B-19 was found in the one patient. ECG findings such as sinus tachycardia with ST-T changes, premature ventricular contraction and complete heart block were found in VMC patients. The predominant echocardiographic features in the patients with VMC were a low ejection fraction, mitral regurgitation and severe pericardial effusion with hypotension. AST and LDH abnormal increase were found in the some patient. **Conclusions** Our findings highlight the importance of identification of VMC in children with clinical suspicion of myocarditis. The present study emphasizes the importance of PCR-based assays for detection of viral agents in myocarditis patients with symptoms of virus infection.

Background

Myocarditis is a potentially life-threatening disease that primarily affects children and young adults with a wide range of symptoms and presentations. Acute myocarditis is considered as one of the most challenging diagnosis in cardiology and can lead to arrhythmia or even sudden cardiac death. Therefore, early diagnosis of myocarditis is important because it is a serious disease [1-3].

Viral infections are one of the most common etiological agents of myocarditis and cardiomyopathy. Viral myocarditis (VMC) is an important cause of morbidity and mortality in different age groups from infants to the elderly, but most occur in children and adults younger than 40 years [4, 5]. VMC is the most common cause of inflammatory heart disease and its incidence is tending to increase further in the twenty-first century [6, 7].

The cardiotropic viruses that cause myocarditis are common viral agents in human infectious diseases. Recently, given the increasing incidence of VMC and the improvement in virus detection methods, research indicated that the spectrum of causing agents VMC has greatly changed [2, 8]. Nowadays, enteroviruses (especially coxsackieviruses B), adenoviruses, human herpes virus 6 (HHV6), parvovirus B19 (PVB19), influenza A and B viruses, and hepatitis viruses are considered to represent common causes of VMC [6, 9].

Recent advances in molecular biology have made it possible to diagnose and manage human infectious diseases. More recently, polymerase chain reaction (PCR) assays has been most widely used in the rapid detection of viral agents in various tissues and body fluids. These methods now are utilized to identify several viruses in different clinical samples of VMC patients [2, 5, 10].

Given that viral myocarditis has become an increasingly common heart disease that endangers human health, the aim of this study was diagnosis of viral myocarditis in children with clinical suspicion of myocarditis by PCR-based assays.

Methods

Patient Population and samples

In this cross-sectional research, the study population consisted of 19 consecutive patients admitted to the Birjand Vali-Asr hospital with clinical suspicion of myocarditis (MCI) who had evidence of microbial infections (Such as diarrhea, cutaneous rash and coryza) in recent weeks from October 2014 to March 2019. The study was approved by the Birjand University of Medical Sciences ethics committee. In addition, all patients' parents signed informed consent and hospital consent forms. Inclusion criteria for the study included initial presenting symptoms of acute cardiovascular collapse, acute CHF with or without ventricular tachycardia, cardiac arrhythmias and evidence of cardiac dysfunction using noninvasive methods.

The patient samples evaluated in this study for the viral myocarditis diagnosis by multiplex Real-time PCR consisted of blood or pericardial fluid.

Clinical and Laboratory Assessment

Echocardiographic criteria used in the diagnosis of acute myocarditis included left ventricular (LV) dysfunction (Ejection Fraction and Fractional Shortening) and ventricular dilation calculated from M-mode echocardiograms [5]. Doppler and color Doppler were used to determine atrioventricular valve regurgitation.

Physical examination, routine electrocardiogram (ECG) and dynamic ECG (Holter) examinations, blood pressure, cardiac monitoring, laboratory testing (Myocardial enzymes and troponin levels), and echocardiographic evaluation were performed and evaluated by an experienced team of cardiologists.

Molecular Detection of Viral Myocarditis

Isolation of nucleic acids was done using the QIAGEN QIAamp DNA Extraction Kit (Qiagen, Germany) as recommended by the company.

Multiplex Real-time PCR by Neuro kit (Bioactiva Diagnostica, Germany) after isolation of nucleic acids was used for detection of Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Adenoviruses (ADV), Herpes

simplex virus 1 and 2 (HSV1, HSV2), Varicellazoster virus (VZV), Enteroviruses, Parechovirus, Human herpes virus 6 and 7 (HHV6, HHV7), and Parvovirus B-19.

Results

The present study was conducted on a total of 19 patients with suspected myocarditis who had evidence of microbial infections in recent weeks admitted to the Birjand Vali-Asr hospital. The ages of the patients ranged from 1 day to 9 years with the mean age of 24.61 ± 4.12 months.

Prodromal symptoms in the patients were fever, cough, abdominal pain, dyspnea, tachypnea, tachycardia, intercostal retraction, cyanosis, nasal flaring, poorfeeding, edema, vomiting, skin rash and diarrhea. The main clinical manifestations were fatigue, pale, respiratory distress, chest pain, precordial discomfort, palpitations, periorbital edema, abdominal discomfort. By physical examination hepatomegaly, abdominal distention and ascites, subcostal and intercostal retraction, hypotension, low heart sound blunt and all kinds of arrhythmia, including premature ventricular, atrial contraction and bradycardia can be found.

Detection of viral genomes in the samples

The results indicated that using multiplex Real-time PCR for differential detection of viral genomes in blood or pericardial fluid samples, 4 out of 19 (21.05%) patients with MCI were found to be virus positive (Table 1). The viruses that were detected in the patients included HHV-6/7, Parvovirus B-19, EBV, VZV and Adenoviruses. It is noteworthy that among the patients with VMC, a co-infection of HHV-6/7 and Parvovirus B-19 was found in the one patient.

Clinical Manifestations of VMC patients

The clinical characteristics of the VMC patients are presented in Table 1. Fatigue and exertional dyspnea were reported by all patients with VMC. The chest X-Ray findings showed that an increase in cardiothoracic ratio (CTR) was found in all patients, whereas the pulmonary vascular marking (PVM) was normal in three cases with VMC.

ECG findings such as sinus tachycardia with ST-T changes were reported in one patient with VMC. Furthermore, a premature ventricular contraction (PVC), complete heart block (CHB) or only ST-T changes was found in one of the three remaining patients. 24 hours Holter-ECG monitoring in one of the patients revealed one episode of ventricular tachycardia running.

The predominant echocardiographic findings in the VMC patients were a low ejection fraction (decrease in systolic LV function) in two patients. Moreover, mitral regurgitation was reported in two cases. Echocardiography revealed severe pericardial effusion with hypotension in only one of the patients.

Laboratory Examination of VMC patients

VMC laboratory test results are shown in Table 1. The results showed the abnormal rate of myocardial enzymes in some the VMC patients. In this study, AST abnormal increase was found in three VMC patients and the LDH abnormality in the one patient. While, there was no abnormal raise in CK-MB and CK enzymes in the VMC patients. It is noteworthy that the troponin assay was negative in all patients.

Treatment and Therapeutic Outcome

The VMC patients were treated by an anti-heart failure medication regimen and IVIG treatment for viral disease. It is noteworthy that the prognosis of treatment in our patients was acceptable given the relatively high age at the time of diagnosis. So, only one patient needed pacemaker implantation and the rest achieved normal functionality in the long-term follow-up.

Discussion

Viral myocarditis (VMC) is one of the common heart diseases to endanger human health in different age groups, especially children. The VMC incidence is tending to increase further in the twenty-first century [5-7].

In the present study a total of 19 clinically suspected myocarditis patients with clinical evidence of microbial infections in recent weeks. The main clinical manifestations of the patients were fatigue, respiratory distress, chest pain, precordial discomfort, palpitations, periorbital edema and abdominal discomfort. Moreover, hepatomegaly, abdominal distention and ascites, subcostal and intercostal retraction, hypotension, low heart sound blunt and all kinds of arrhythmia, including premature ventricular, atrial contraction and bradycardia can be reported on physical examination.

The results of using multiplex Real-time PCR for differential detection of viral genomes in blood or pericardial fluid samples indicated that 21.05% patients with myocarditis (MCI) were found to be virus positive. In the study of Jeserich et al. [2] in Germany using nested RT-PCR, 38% of peripheral leukocytes and/or plasma samples of MCI patients were virus positive. In another study, the viral nucleic acids were detected in ventricular and peripheral serum, and Endomyocardial biopsy (EMB) samples of 14%, 20%, and 46% patients with MCI, respectively [4]. In general, the incidence of VMC varies in different studies appear to depend on the geographical distribution, various virus identification methods and sample sites.

Recently, studies indicated that the viral spectrum of VMC has greatly changed with the rising VMC incidence rate and continuous improvement in virus detection methods [2, 6]. The spectrum of viruses that were detected in our study (HHV-6/7, Parvovirus B-19, EBV, VZV and Adenoviruses) was comparable with the results of previously published studies. In Pawlak and colleague study [4], PB19V, human enteroviruses (HEV), human adenovirus (HAdV), and HHV-6 were detected in samples of patients with myocarditis. Another study demonstrated that the most prevalent virus infection in MCI patients was EBV infection followed by HHV-7. In addition, one patient was found to be PVB19 positive and HHV-6 variant B genome was detected in another patient [2]. Griffin found the viral spectrum of VMC in myocardial specimens was mostly HAdV, followed by HEV, as well as CMV and HSV [11]. Finally, VMC caused by

multiple viruses is also more common than before [6, 12]. The results of our study showed that among the patients with VMC, a co-infection of HHV-6/7 and Parvovirus B-19 was found in the one patient. Jeserich et al [2] also reported two VMC patients with co-infections of EBV and HHV-7.

At present, the ECG and Holter monitoring are still one of the important indicators of VMC [13, 14]. In our study, ECG findings such as sinus tachycardia, ST-T changes, premature ventricular contraction (PVC) and complete heart block (CHB) were reported patient with VMC. Furthermore, 24 hours Holter-ECG monitoring in one of the patients revealed one episode of ventricular tachycardia running. Similar to our study, the results of a previous study showed that ECG findings such as new atrial or ventricular premature beats were found in 62% of VMC patients, new right or left bundle block in two virus positive patients, and new atrial fibrillation and ST-T changes in three patients [2]. Niu and et al. [13] also revealed that ECG examination and 24 hours dynamic ECG was positive in 88.71% and 92.31% children with viral myocarditis, respectively.

Finally, the results of the present study showed that the AST abnormal increase was found in three VMC patients and the LDH abnormality in the one patient. While, there was no abnormal increase in CK-MB and CK enzymes in the patients. In a previous study, the abnormal rate of myocardial enzymes was reported in 96.77% of VMC patients that CK-MB abnormal increase was more common, followed by LDH anomaly [13]. Although elevated cardiac enzymes can be present in myocarditis, but studies have shown that the increase in myocardial enzymes is non-specific and these enzymes can be elevated due to other conditions [6, 15]. Moreover, cardiac troponins are well established as the best indicators of myocardial injury [6, 16]. In our study, the troponin assay was negative in all VMC patients. In Smith and colleague study [17], cardiac troponin I values were elevated in 18 (34%) of 53 patients with myocarditis. In another study, an elevated cardiac troponin level was reported in all patients with myocarditis [18]. It is noteworthy that the cardiac troponin levels depend on numerous factors such as time of measurement and severity of myocardial injury. In addition, cardiac troponin in serum is not stable and can be affected by endogenous material.

Conclusions

Given that viral myocarditis is increasingly common heart disease to endanger human health, our findings highlight the importance of identification of VMC in children with clinical suspicion of myocarditis. The present study emphasizes the importance of PCR-based assays for detection of viral agents in MCI patients with symptoms of virus infection.

Abbreviations

VMC: Viral myocarditis; PCR: Polymerase Chain Reaction; HHV6: Human Herpes Virus 6; MCI: Myocarditis; LV: Left Ventricular; ECG: Electrocardiogram; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; ADV: Adenoviruses; VZV: Varicellazoster virus; CTR: Cardiothoracic ratio; PVM: Pulmonary Vascular Marking; EF: Ejection Fraction; FS: Fractional Shortening; PVC: Premature Ventricular Contraction; VT:

Ventricular Tachycardia; CHB: Complete Heart Block; MR: Mitral Regurgitation; TR: Tricuspid Regurgitation; PE: Pericardial Effusion.

Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contribution

FS designed the study, collected the data, and revised the manuscript. SHS collected the data and revised the manuscript. MY wrote the draft manuscript and edited the paper. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

The study was approved by the Birjand University of Medical Sciences ethics committee. All patients' parents signed informed consent and hospital consent forms.

Consent for publication

No applicable.

Competing interests

The authors declare that they have no competing interests.

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Table

Table 1. Clinical manifestations and laboratory findings of children with viral myocarditis

Manifestations	Chest X-Ray	ECG		Myocardial enzyme					Echo findings	Virus detection
		ECG	HOLTER	CK- MB	CK	LDH	AST, ALT	Troponin		
Failure, exertional dyspnea, Tachycardia	CTR: increased PVM: NL	Sinus Tachycardia, ST-T change	Sinus Tachycardia	24	98	476	59	Negative	EF=31% FS=16%	HSV6-7 Parvovirus B- 19
Tachycardia, dyspnea, Poorfeeding (Fatigue), Lethargy, Fever, Skin rash	CTR: increased PVM: increased	ST-T change	ST-T change	-	20	397	41, 40	Negative	EF=74% FS=25% PE (Size=17MM)	EBV
Abdominal pain, Vomiting, Hepatomegaly, Respiratory dyspnea, Hypotension	CTR: increased PVM: NL	PVC	VT RUN	-	170	520	275, 35	Negative	EF=27% FS=13% MR (PG=65mmHg) TR (PG=32mmHg)	VZV
Bradycardia, Decreased systolic blood pressure, Murmur, Dyspnea, Exertional dyspnea	CTR: increased PVM: NL	CHB	CHB & Bradycardia	-	22	345	37, 40	Negative	EF=55% FS=25% MR (PG=72mmHg)	Adenoviruses

CTR: Cardiothoracic ratio, PVM: Pulmonary Vascular Marking, EF: Ejection Fraction, FS: Fractional Shortening, PVC: Premature Ventricular Contraction, VT: Ventricular Tachycardia, CHB: Complete Heart Block, MR: Mitral Regurgitation, TR: Tricuspid Regurgitation, PE: Pericardial Effusion.