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Diagnosis of viral myocarditis in children by multiplex real-time PCR in Vali-Asr Hospital, Birjand, eastern Iran

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ABSTRACT

Background and Objective: 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 to diagnose VMC in children with clinical suspicion of myocarditis (MCI) using multiplex real-time PCR in Birjand Vali-Asr Hospital.

Methods: This cross-sectional study was conducted on 19 patients with clinical suspicion of MCI, who had evidence of infections in the recent weeks. Routine electrocardiogram and dynamic electrocardiogram (ECG) examinations, auxiliary laboratory testing and echocardiographic evaluation were performed. Multiplex real-time PCR was used for detection of viral agents in blood or pericardial fluid samples.

Findings: The results indicated that 4 out of 19 (21.05%) MCI patients were virus-positive. The spectrum of viral agents included human herpesvirus 6, 7 (HHV), parvovirus B-19 (PVB19), Epstein–Barr virus (EBV), varicella zoster virus (VZV) and adenoviruses. A co-infection of HHV-6, 7 and PVB19 was found in one patient. The ECG findings such as sinus tachycardia with ST-T changes, premature ventricular contraction and complete heart block were observed in VMC patients. The predominant echocardiographic features in VMC patients were low ejection fraction, mitral regurgitation and severe pericardial effusion with hypotension. Aspartate aminotransferase (AST) and lactic dehydrogenase (LDH) had abnormal increase in some patients.

Conclusion: Our findings highlight the importance of identification of VMC in children with clinical suspicion of MCI. The present study emphasizes the importance of PCR-based assays for detection of viral agents in MCI patients with symptoms of virus infection.

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Introduction

Myocarditis (MCI) is a potentially life-threatening disease that primarily affects children and young adults with a wide range of symptoms and presentations. Acute MCI is considered as one of the most challenging diagnosis in cardiology and can lead to arrhythmia or even sudden cardiac death. Therefore, the early diagnosis of MCI is important because it is a serious disease [1- 3]. Viral infections are one of the most common etiological agents of MCI 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]. The 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 MCI are common viral agents in human infectious diseases. Recently, given the increasing incidence of VMC and improvement in virus detection methods, research has indicated that the spectrum of causing agents VMC has greatly changed [2, 8]. Nowadays, enteroviruses (especially coxsackieviruses B), adenoviruses (ADVs), 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 have 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].

The VMC is rare in children, but seemingly becoming more common, and its real incidence in the general population is unknown [11, 12]. A few studies have reported the incidence of VMC in children. Silva et al. reported VMC in a five-year-old child with positive serology for PVB19 and Epstein-Barr virus (EBV) [11]. Another study showed a possible association of rhinovirus C and MCI in a 4.5-year-old child presenting with acute onset of dilated cardiomyopathy [8]. Therefore, the need for the study of VMC incidence in children is greater than ever.

Given that the VMC becomes an increasingly common heart disease that threats human health, the aim of this study was to diagnose VMC in children with clinical suspicion of MCI through PCR-based assays.

Methods

Patient Population and samples

In the current study, the population included 19 patients with suspected MCI and symptoms of microbial infections (diarrhea, cutaneous rash and coryza) in the recent weeks, referred to the Vali-Asr Hospital in Birjand. This study was performed during 5 years (from October 2014 to March 2019) due to the rarity of MCI in children and considering the population of Birjand city. Early clinical diagnosis of MCI was performed by a pediatric cardiologist. Blood and pericardial fluid samples were taken from children with suspected MCI for further investigation. The study was approved by the Ethics Committee of Birjand University of Medical Sciences (IR.BUMS.REC.1398.242). In addition, informed consent and hospital consent forms were signed by all parents of children. Inclusion criteria for the study were initial presenting symptoms of acute cardiovascular collapse, acute congestive heart failure with or without ventricular tachycardia, cardiac arrhythmias and diagnosis of cardiac dysfunction through noninvasive methods.

Clinical and Laboratory Assessment

Echocardiographic criteria used in the diagnosis of acute MCI included left ventricular (LV) dysfunction (ejection fraction and fractional shortening) and ventricular dilation calculated from M-mode echocardiograms [5]. Doppler and color Doppler were applied to determine atrioventricular valve regurgitation.

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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 carried out and evaluated by an experienced team of cardiologists.

Molecular Detection of Viral Myocarditis

Blood and pericardial fluid samples taken from patients using multiplex Real-time PCR were evaluated to diagnose VMC. Extraction of nucleic acids was done through the QIAGEN QIAamp DNA Extraction Kit (Qiagen, Germany) and QIAGEN QIAamp Viral RNA Mini Kit (Qiagen, Germany) as recommended by the company. The total amount of extracted RNA was immediately transcribed to cDNA using SuperScript III Reverse Transcriptase (Invitrogen, USA) according to the manufacturer's instructions.

After nucleic acid extraction, the multiplex real-time PCR method using Neuro kit (Bioactiva Diagnostica, Germany) was applied to detect cytomegalovirus (CMV), EBV, ADVs, herpes simplex virus 1 and 2 (HSV1/2), varicella-zoster virus (VZV), enteroviruses, parechovirus, HHV6/7 and PVB19.

Statistical analysis

The data were analyzed using SPSS 21. Frequency and percentages were calculated as well as the Pearson chi-square and Fisher's exact tests were used. Moreover, $p< 0.05$ was statistically regarded as significant level.

Results

The present study was conducted on 19 patients with suspected MCI and symptoms of microbial infections in the recent weeks, referred to the Vali-Asr Hospital in Birjand. The ages of the patients ranged from 1 day to 9 years with the mean age of 24.61±4.12 months. There were 11 (57.9%) males and 8 (42.1%) females.

Prodromal symptoms in 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 and abdominal discomfort. By physical examination, the 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 were found.

Detection of viral genomes in the samples

The results indicated that by using multiplex real-time PCR for differential detection of viral genomes in blood or pericardial fluid samples, it was observed that 4 out of 19 (21.05%) patients with MCI were virus-positive (Table 1). The viruses detected in patients included HHV-6/7, PVB19, EBV, VZV and ADVs. The EBV was detected from pericardial fluid sample of one patient and other viruses from blood samples. It is noteworthy that among the patients with VMC one patient had a co-infection of HHV-6/7 and PVB19.

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 VMC patients. The chest X-Ray findings showed an increase in cardiothoracic ratio (CTR) of all patients, whereas the pulmonary vascular marking (PVM) was normal in three cases with VMC.

The 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 were found in one of the three remaining patients. Moreover, 24-hour Holter-ECG monitoring in one of the patients revealed one episode of a run of ventricular tachycardia.

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The predominant echocardiographic findings in 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

The VMC laboratory test results are illustrated in table 1. The results indicated the abnormal rate of myocardial enzymes in some VMC patients. In the ongoing study, the abnormal increase of AST was found in three VMC patients, and the abnormal lactic dehydrogenase (LDH) was observed in one patient. However, there was no abnormal raise in CK-MB and CK enzymes in 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 intravenous immunoglobulin. It is noteworthy that the prognosis of treatment in our patients was acceptable given the relatively high age at the time of diagnosis. Thus, only one patient needed pacemaker implantation and the rest achieved normal functionality in long-term follow-up.

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

Clinical Manifestations	Chest X-Ray	ECG		Myocardial enzyme					Echo findings	Virus detection
		ECG	HOLTER	CK-MB	CK	LDH	AST, ALT	Troponin		
Case 1 (Male/ 8 years) Growth failure, exertional dyspnea, Tachycardia	CTR: increased PVM: NL	Sinus Tachycardia, ST-T change	Sinus Tachycardia	24	98	476	59	-	EF=31 % FS=16%	HHV6-7 Parvovirus B-19
Case 2 (Male/ 5 months) Tachycardia, Dyspnea, Poorfeeding (Fatigue), Drowsiness, Fever, Skin rash	CTR: increased PVM: increased	ST-T change	ST-T change	-	20	397	41, 40	-	EF=74 % FS=25% PE (Size=17MM)	EBV
Case 3 (Male/ 9 years) Fever, Abdominal pain, Vomiting, Hepatomegaly, Exertional dyspnea, hypotension	CTR: increased PVM: NL	PVC	VT RUN	-	170	520	275, 35	-	EF=27 % FS=13% MR (PG=65 mmHg) TR (PG=32 mmHg)	VZV
Case 4 (Female/ 7 years) Bradycardia, Mid systolic Murmur, Fatigue, Exertional dyspnea	CTR: increased PVM: NL	CHB	CHB & Bradycardia	-	22	345	37, 40	-	EF=55 % FS=25% MR (PG=72 mmHg)	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.

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Discussion

The present study was carried out on 19 patients with suspected MCI and symptoms of microbial infections in the recent weeks. 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 MCI were 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 14%, 20%, and 46% patients with MCI in their ventricular serum, peripheral serum and endomyocardial biopsy (EMB), respectively [4]. In general, the incidence of VMC varies in different studies because of the geographical distribution, various virus identification methods and sample sites.

Recently, various studies have suggested that the viral spectrum of VMC has greatly changed with rising VMC incidence rate and continuous improvement in virus detection methods [2, 6]. The spectrum of detected viruses (HHV-6/7, PVB19, EBV, VZV and ADVs) in the current study was comparable with those of previous studies. Pawlak et al.[4] found PB19V, human enteroviruses (HEV), human adenovirus (HAdV), and HHV-6 in the samples of MCI patients. Another study demonstrated that the most prevalent virus infection in MCI patients was EBV infection followed by HHV-7. In addition, one patient had PVB19 positive, and the HHV-6 variant B genome was detected in another patient [2]. Griffin et al. found the viral spectrum of VMC in myocardial specimens was mostly HAdV, followed by HEV, CMV and HSV [13]. Finally, the VMC caused by multiple viruses is also more common than before [6, 14]. The results of the present study exhibited that among patients with VMC, a co-infection of HHV-6/7 and PVB19 was found in one patient. Besides, Jeserich et al. [2] reported two VMC patients with confections of EBV and HHV-7.

At present, the ECG and Holter monitoring are still one of the important indicators of VMC [15, 16]. In the current study, the ECG findings such as sinus tachycardia, ST-T changes, PVC and CHB were reported for VMC patient. Furthermore, 24-hour Holter-ECG monitoring in one of the patients revealed one episode of a run of ventricular tachycardia. Similar to our study, the results of a previous study displayed that the 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 as well as new atrial fibrillation and ST-T changes in three patients [2]. Niu and et al. [15] also revealed that the ECG examination and 24-hour dynamic ECG were positive in 88.71% and 92.31% children with VMC, respectively.

Finally, the results of the ongoing study suggested that the abnormal increase of AST was found in three VMC patients, and the abnormal LDH was observed in one patient. Nevertheless, there was no abnormal increase in CK-MB and CK enzymes in patients. In a previous study, the abnormal rate of myocardial enzymes was reported in 96.77% of VMC patients so that the abnormal increase of CK-MB was more common, followed by LDH anomaly [15]. Although elevated cardiac enzymes can be present in MCI, different studies have shown that the increase in myocardial enzymes is non-specific, and these enzymes can be elevated due to other conditions [6, 17]. Moreover, cardiac troponins are well established as the best indicators of myocardial injury [6, 18]. In the current study, the troponin assay was negative in all VMC patients. Smith et al. [19], suggested that the cardiac troponin I values were elevated in 18 (34%) of 53 patients with MCI. In another study, the elevated cardiac troponin level was reported in all MCI patients [20]. It is noteworthy that the cardiac troponin levels depend on numerous factors including time of measurement and severity of myocardial injury. Besides, cardiac troponin in serum is not stable and can be affected by endogenous material.

The relative small size of population and lack of data on cardiac magnetic resonance (CMR) imaging and myocardial biopsy specimens were the limitations of the current study. Furthermore, viral nucleic acid testing alone could not determine the duration of infection. Hence, it is necessary to perform more prospective study with a larger population through combining serological and molecular biology assays to detect the VMC as well as to validate these findings.

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

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Ethics approval

The study was approved by the Ethics Committee (IR.BUMS.REC.1398.242) of Birjand University of Medical Sciences. In addition, informed consent and hospital consent forms were signed by all parents of children.

Author contributions

Forod Salehi designed the study, collected the data and revised the manuscript. Shiva Salehi collected the data and revised the manuscript. Masoud Yousefi wrote the draft manuscript and edited the paper. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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