Enteroviruses in Acute Myocardial Infarction

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Abstract

Background: Human enteroviruses (EVs) may have a role as a possible risk factor in the pathogenesis of MI. The aim of this study was to evaluate the presence of enterovirus genomic RNA in peripheral blood samples of patients with acute myocardial infarction (MI).

Methods: We investigated the presence of enterovirus genomic RNA in the peripheral blood of 115 patients with acute MI hospitalized in the Coronary Care Unit of Imam Reza and Ghaem University Hospitals (Mashhad, Iran) by RT-PCR using the virus specific primers.

Results: The subjects' mean (±SD) age was 63.5 (±9.4) years (range: 38-82) and 38.3 % of the subjects were female. Of 115 patient specimens, 3 (2.6%) were positive in RT-PCR.

Conclusion: The prevalence of enterovirus in MI patients is considerable. More investigations are needed to determine the causal role of enteroviruses in MI.

Keywords: Myocardial infarction, Enteroviruses, Polymerase chain reaction

Introduction

Myocardial infarction (MI) is a main cause of mortality worldwide and results from an acute coronary syndrome (1). Several studies suggested that some infectious agents including viruses are associated with the pathogenesis of atherosclerosis and MI (2,3). Furthermore, several reports suggested a potential role of human enteroviruses (EVs) as a possible risk factor in the pathogenesis of MI (4-6). A large group of viruses are classified as enteroviruses which transmitted by fecal-oral contamination and respiratory droplets. Enteroviruses are belonging to Picornaviridae family which is non-enveloped RNA viruses about 30 nm in diameter. The viruses can infect human worldwide and cause variety of diseases, including non specific viral illnesses to potentially serious fetal dis-

eases (7). Enteroviruses are the major cause of myocarditis (8). Increasing in antibody titers against coxsackiviruses was showed in patients with MI (9) and suggested an association between enterovirus infection and MI (6).

The aim of present study was to evaluate the presence of enteroviruses genomes in patients with acute MI.

Materials and Methods

Subjects

This study was conducted in Mashhad University of Medical Sciences, Mashhad, Iran. We analyzed the presence of enteroviruses genomic RNA in the peripheral blood of 115 patients 38-82 years of

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age with acute MI hospitalized in the coronary care unit of Imam Reza and Ghaem University Hospitals. MI diagnostic criteria were including past history of MI, symptoms at onset, maximum levels of serum enzymes and relevant electrocardiograms (10). Approximately 5 ml blood sample was collected in EDTA tubes for RT-PCR testing within the first 12 hours after their hospitalization.

RNA extraction and RT-PCR

RNA was extracted from 140 µl of sera by using the QIAamp viral RNA mini kit (Qiagen Company, Valencia, USA) according to the manufacturer's recommended procedure and eluted at total volume of 60 µl elusion buffer. For cDNA synthesis, reverse transcription was performed using Omniscript RT kit (Qiagen Company, Valencia, USA) according to the manufacturer's instruction. In addition, RNA was extracted from polio vaccine and its cDNA was prepared by the same protocol and it was used as positive control in our experiment. PCR was done as described previously with minor modifications (11); briefly, PCR mixture consisted of 20 pmol of F1(5'-CAAGCACTTCTGTTTCCCCGG-3') and F2 (5'-TCCTCCGGCCCCTGAATGCG-3') primers and 40 pmol of R (5'-ATTGTCACCATAAG-CAGCCA-3') primer, 3.5 mM $MgCl_2$, 0.2 mM each dNTP, 3U Taq polymerase (Cinnagen, Tehran, Iran) in a total reaction volume of 25 µl. Amplification was carried out for 40 cycles (94°C for 1 min, 42°C for 1 min, 72°C for 2 min) after an initial denaturation step in 94°C for 5 min. The cycles were followed by a 5 min extension at 72°C and the PCR product was visualized on a 2% agarose gel by Green-Viewer staining.

Results

In our study 115 patients (71 male and 44 female; 63.5 ± 9.4 years) were subjected for detection of enteroviruses by PCR. The difference of positive samples in two genders and also in different aged groups were not statistically significant ($P= 0.999$) (Table1 and 2).

Table 1: Results of PCR according to the gender

PCR result Gender	Negative		Positive		Total	
	n	%	n	%	n	%
Female	43	38.40	1	33.34	44	38.26
Male	69	61.60	2	66.66	71	61.74
Total	112	100	3	100	115	100
Statistical Test	Fisher's Exact Test			*P*-Value=0.999*		

*: Statistically not significant

Table 2: Results of PCR according to the age groups

PCR result Age (year)	Negative		Positive		Total	
	n	%	n	%	n	%
≥50	12	10.71	0	0	12	10.43
51-60	29	25.89	1	33.33	30	26.09
61-70	38	33.93	1	33.33	39	33.91
≤70	33	29.47	1	33.34	34	29.57
Total	112	100	3	100	115	100
Statistical Test	Fisher's Exact Test			*P*-alue=0.999*		

*: Statistically not significant

In PCR amplification method for positive control sample, PCR product of 440 bp and 155 bp were obtained using F1/R and F2/R primer sets, respectively. Of 115 patient specimens, 3 (2.6%) were positive in PCR using specific primers; however, they only showed a 440 bp fragment in PCR amplification (Fig. 1).
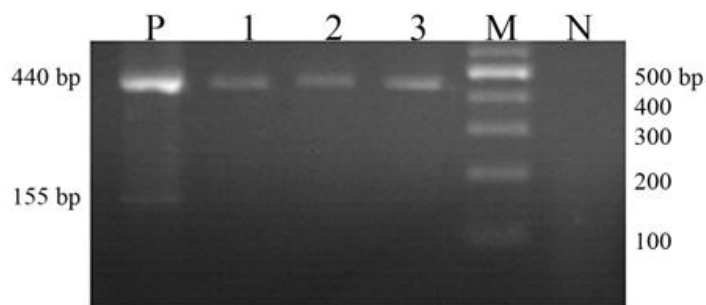

Fig. 1: Gel electrophoresis of PCR products. Semi nested RT-PCR was performed for amplifying 440 bp and 155 bp fragments using specific primers located in the 5´-noncoding region of the enterooviral genomic RNA sequences. Lanes 1 to 3 correspond to enterovirus positive RT-PCR; M= 100 bp DNA size marker; P= positive control; N= negative control of the PCR

Discussion

Myocardial infarction (MI) is associated with inflammatory responses which could be initiated by infectious agents (4, 12). Several previous studies showed that different bacterial and/or viral infections including enterovirus infections may associate with the incidence of coronary heart disease and elevate the risk of MI (3). The possible role of human enteroviruses especially coxsackievirus B (CV-B) in the pathogenesis of MI has been proposed (5, 13). CV-B serotypes showed a strong cardiac tropism. It can lead to persistent infection of human monocytes and vascular endothelial cells as well. Protease 2 of the virus is capable to cleave dystrophin, leading to EV-induced cardiac pathologies (4, 14-16). The exact mechanism by which enterovirus may induce atherosclerosis or MI is not established.

Several studies investigated the presence of enteroviruses or their specific antibodies in patients with MI. Roivainen et al. investigated the enterovirus group-specific immunoglobulin G (IgG) antibody on sera of patients with MI and they showed the high level of enterovirus group specific antibodies in the studied subjects. They also showed age-dependence of the association that the risk of MI was high in men aged 25 to 49 years; however, they concluded the high level of the antibodies may be due to frequently exposure to enteroviruses in the past and it may increase the risk of MI in men with normal serum cholesterol (6). In our study, we analyzed the presence of enteroviruses genome in blood samples of patients with MI by PCR. In spite of serum antibodies which may show the past infection, PCR-based detection methods are able to revealed the viral genome as a marker of the presence of the virus in patient bloods. In our study, the mean age of the patients was 63.5 and only 10% of the studied patients were aged ≤50 years. There was no statistically significant difference in different age groups which our results did not agree with a previous study (6). Reunanen et al. investigated the antibodies against several infectious agents including enteroviruses as a predictor for MI and they showed the high levels of antibodies to enteroviruses is associated with the risk of coronary heart disease in men without baseline history of heart disease (3). In addition, several studies showed that enterovirus infections were associated with acute MI in both uncontrolled and controlled clinical series (17-19); however, other studies did not support the association (20). Andreoletti et al. investigated the presence of enteroviruses RNA sequences using RT-PCR and capsid viral protein 1 (VP1) and the virus-mediated focal disruption of dystrophin by immunohistochemistry assays in endomyocardial tissues of patients who died suddenly of acute MI and they compared the patient results with similar samples of control group. They showed that the active cardiovascular infection of coxsackievirus B is higher in case group than the control matched subjects and they concluded the EVs may have a role in pathogenesis of acute MI (4).

In conclusion, the presence of EVs 5´-non coding sequence of the genome was detected in 2.6% of patients' blood samples at the first 12 hours after heart attack which may show the potential role of EVs in the pathogenesis of MI; however, more controlled studies are needed for confirming the role of EVs in MI.

Ethical considerations

Acknowledgments

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