Comparison of the prevalence of enteroviruses in blood samples of patients with and without unstable angina

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Original Article

Abstract

BACKGROUND: Although the role of enteroviruses has been proved in heart diseases, extensive information is not available on the association between enteroviruses and unstable angina. In the present study, the authors compared the prevalence of enteroviruses in patients with and without unstable angina.

METHODS: Blood samples were taken from 51 patients with unstable angina and 55 patients without unstable angina or myocardial infarction that were admitted to Imam Reza and Ghaem hospitals (Mashhad, northeast of Iran). Reverse transcription polymerase chain reaction (RT-PCR) was performed using specific primers for the detection of the enteroviruses in blood samples of study subjects.

RESULTS: Patients with and without unstable angina were similar in age with mean ± standard deviation of 62.6 ± 12.8 and 59.7 ± 12.7 years, respectively (P = 0.243) and there were no differences in gender in these two groups (P = 0.174). Prevalence of the enteroviruses in patients with unstable angina was higher only in 66-80 years age group compared to the control group (patients without unstable angina, P = 0.032). There was a higher prevalence of enterovirus RNA positivity in the blood samples of women with unstable angina (75.9%) than those without unstable angina (41.7%, P = 0.011), however, no significant difference was observed in men (P = 0.983).

CONCLUSION: Our data showed that enteroviral RNA positivity was higher in patients with unstable angina compared to those without unstable angina. However, the differences between the two groups were not statistically significant.

Keywords: Unstable Angina, Enterovirus, Reverse Transcriptase PCR

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Introduction

Ischemic heart disease is the number one cause of mortality all around the world. Coronary artery disease may be manifested clinically as either stable ischemic heart disease (SIHD) or an acute coronary syndrome (ACS). The latter can be subdivided into two groups. The first sub-group is patients with acute ST-elevation myocardial infarction (STEMI),

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and the second sub-group is patients with unstable angina (UA) and non-ST-segment elevation myocardial infarction (NSTEMI).[1]

Compared to STEMI, the prevalence of UA/NSTEMI is increasing. Almost half of the patients with UA/NSTEMI are women, while more than three-fourths of patients with STEMI are men. Patients with UA/NSTEMI may face a wide range of early risks (first 30 days) that varies from 2 to 10 percent.[2]

UA stems from imbalance between the myocardial oxygen supply and demand. Rupture of unstable atheromatous plaque with superimposed non-occlusive thrombi may be the main etiology in patients with UA. Inflammation and/or infection may play a role for thrombogenesis in these patients.[3] There is some evidence indicating a relationship between bacteria or viruses [such as herpes simplex virus (HSV), cytomegalovirus (CMV), and Helicobacter pylori (HP)] and atherosclerosis or myocardial infarction (MI).[4,5]

It has been found that the enteroviruses infections are related to myocarditis. Furthermore, the experimental animal models have provided evidence that enterovirus infections may cause atrial fibrillation.[6] The enterovirus myocarditis in infancy leads to greater mortality and increases the risk of coronary heart disease in the future.[7] Enteroviruses are endemic. Most people are exposed to various serotypes of the enteroviruses during their lifetime. Enteroviruses are the most common cause of acute and chronic viral myocarditis.[8]

Transmission of the enterovirus infection is through fecal-oral route and respiratory system. They are non-enveloped positive sense RNA virus belonging to Picornaviridae family.[9] Enteroviruses include five subgroups including polioviruses, coxsackieviruses (A and B), echoviruses, and novel types of the enteroviruses. Over 70 serotypes of the enteroviruses have been isolated from human. Some types of human enteroviruses are associated with heart diseases.[10,11] Enteroviruses may cause asymptomatic disease and they probably appear in healthy people as well.

A few studies have evaluated the relationship between MI and enteroviruses. However, the knowledge in this regard is not yet comprehensive. In the current study, the frequency of the enteroviruses was determined in blood samples of UA patients compared to patients without UA.

Materials and Methods

This prospective cross-sectional study was conducted at the Mashhad University of Medical Sciences, Iran and was confirmed by the clinical ethics committee (approval number 89165). Between March to September 2013, 106 consecutive patients (age range, 35 to 80 years) were enrolled in this study who were admitted to the cardiology ward and other departments of Imam Reza and Ghaem hospitals.

In this research, patients were divided into two groups: patients with UA (n = 51) and patients without UA (n = 55). Inclusion criteria for the first group (UA patients) included women and men between 35-80 years of age and diagnosis of UA, based on the criteria of having ischemic type chest discomfort, electrocardiographic (ECG) changes, evaluation of serum markers and coronary artery involvement in angiography.[3]

We excluded patients with signs of myocardial ischemia in the absence of coronary artery disease, such as high-output heart failure (thyrotoxicosis, anemia, beriberi), vasculitis or inflammatory diseases, inherited metabolic disease, congenital anomalies of the coronary arteries, blood diseases (polycythemia vera, thrombocytosis, hypercoagulable disorders), substance abuse and coronary vascular injury.

The second group (patients without UA) consisted of 55 patients who were hospitalized in other departments of hospitals during the same period. These patients were examined by a cardiologist and those who had cardiovascular risk factors were excluded. Moreover, subjects with UA and MI were excluded.

After obtaining informed consent from the study subjects, 2 ml blood sample from the brachial vein of each subject were collected in Ethylenediaminetetraacetic acid (EDTA) tube, and the tubes were kept on ice. Sera were separated immediately and kept frozen at -70 °C.

RNA extraction was done according to the kit protocol (CinnaGen Co, Tehran, Iran). Complimentary DNA (cDNA) synthesis was performed using reverse transcription method according to the recommendations of Easy cDNA Synthesis Kit (Pars Toos Co, Mashhad, Iran) manufacturers. Poliovirus (Picornaviridae family) vaccine was used as a positive control.

For semi-nested PCR, the following primers were used: F1 (5'- CAAGCACTTCTGTTTCCCCGG-3'), F2 (5'- TCCTCCGGCCCCTGAATGCG-3'), R (5'- ATTGTCACCATAAGCAGCCA-3').[12] PCR method was performed as described previously:[9] PCR mixture consisted of 20 pmol of each primer, 3.5 mM $MgCl_2$, 0.2 mM each dNTP, 4U Taq DNA polythermase (Cinagen, Iran) in a total reaction volume of 25 µl.

Table 1. Demographic information of patients with and without unstable angina

Variables	With unstable angina (n = 51)	Without unstable angina (n = 55)	P
Sex [n (%)]			
Men	22 (43.1)	31 (56.4)	0.174*
Women	29 (56.9)	24 (43.6)	
Age (mean ± SD)	62.6 ± 12.8	59.7 ± 12.8	0.243**

* Pearson chi-square test; ** Student's t-test
SD: Standard deviation

Amplification was performed for 40 cycles: 94 °C for 1 min, 42 °C for 1 min, 72 °C for 2 min and an initial denaturation step in 94 °C for 5 min and a final extension step of 72 °C for 5 min was used. The PCR products were analyzed on 1.5% agarose gel.

Student's t-test was used to compare differences for the quantitative variables such as age. Nominal qualitative variables [(sex and reverse transcription polymerase chain reaction (RT-PCR)] were compared using Pearson's chi-square test. The variables were reported as number (percentage) or mean (± standard deviation) as appropriate. The Fisher's exact test was also used for cases in which 20% of expected frequencies of tables were fewer than five. For statistical analysis, SPSS (version 18, SPSS Inc., Chicago, IL, USA) was used, and P-value less than 0.05 was considered significant.

Results

Table 1 summarizes demographic information of patients with and without unstable angina. The differences between the two groups were not statistically significant, in terms of gender (P = 0.174). The comparison of the mean age of subjects with and without unstable angina was performed with the Student's t-test and no significant difference were found between the two groups (P = 0.243).

It can be seen in figure 1 that in the positive control sample (poliovirus vaccine) and selected enterovirus-positive samples using F1/R and F2/R primers, the sizes of the bands were 440 bp and 155 bp, respectively.

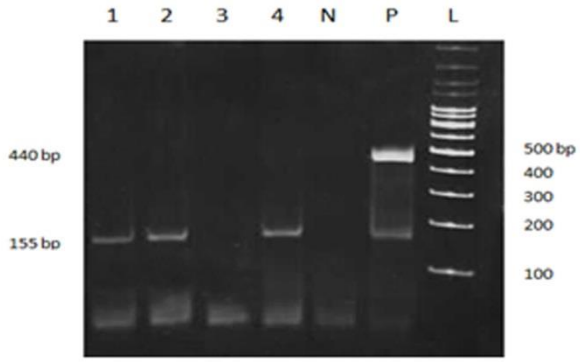

Figure 1. Electrophoresis of polymerase chain reaction (PCR) products
Semi-nested reverse transcription polymerase chain reaction (RT-PCR) was used for amplification of 440 bp and 155 bp fragments, within the 5' transcribed domain of enteroviruses genome, lanes 2, 1 and 4 represent enteroviruses-positive samples and lane 3 represents enterovirus-negative sample
N: Negative control; P: Positive control; L = 100 bp DNA ladder

Table 2 shows the percentages of enterovirus infection in individuals with and without UA, regardless of their age and sex. Among patients with UA, 34 samples were found RT-PCR positive. These results indicate that the rate of enterovirus infection in patients with unstable angina was higher than subjects without unstable angina; however, there was no significant difference between the two groups (P = 0.067).

RT-PCR analysis in men demonstrated that the differences between those with and without UA were not statistically significant (P-value = 0.983). However, significant differences were observed between the two groups of women (P = 0.011, Table 3).

Table 2. Distribution of enterovirus infection in patients with and without unstable angina using reverse transcription polymerase chain reaction (RT- PCR)

Enterovirus infection	With unstable angina [n (%)]	Without unstable angina [n (%)]	Total [n (%)]
RT-PCR-positive	34 (66.7)	27 (49.1)	61 (57.5)
RT-PCR-negative	17 (33.3)	28 (50.9)	45 (42.5)
Statistic test		Pearson chi-square = 3.35	
P		0.067	

RT-PCR: Reverse transcription polymerase chain reaction

Table 3. Detection of enteroviruses in patients with and without unstable angina according to the gender

Sex	Enterovirus RT-PCR	With unstable angina [n (%)]	Without unstable angina [n (%)]	P
Men	Positive	12 (54.5)	17 (54.8)	0.983*
	Negative	10 (45.5)	14 (45.2)	
Women	Positive	22 (75.9)	10 (41.7)	0.011*
	Negative	7 (24.1)	14 (58.3)	

* Pearson chi-square test; RT-PCR: Reverse transcription polymerase chain reaction

Table 4 compares the rate of enterovirus infection among three age groups of patients with and without UA. RT-PCR analysis were statistically significant between the two groups in the range of 66-80 years old (P = 0.032), however it did not reach statistical significance in those in the age range of 35-50 years (P = 0.703) and 51-65 years (P = 0.795).

Discussion

Many studies have considered the association of microorganisms, especially viruses, and heart diseases.[4,11,13] The aim of this study was to compare the prevalence of enterovirus genome in blood samples of patients with UA and a control group.

In the present study, 51 patients with diagnosis of UA and 55 controls without signs and symptoms of UA were screened for the detection of the enterovirus RNA in their blood samples. Patients with and without UA had mean age of 62.6 ± 12.8 and 59.7 ± 12.7 years old, respectively. We have shown in this study that the number of women with UA who had enterovirus infection was significantly higher than women without UA. Moreover, the data has shown that in the age group of 66-80 years old, the number of patients with UA who had enterovirus RNA in their blood samples was significantly higher than the control group. In our study, the enteroviruses were detected in 66.7% of patients with UA and 49.1% of the control group (P-value = 0.067); considering that the P-value is close to 0.05, more extensive research is needed to better clarify the significance of difference.

Compared with other similar studies, the incidence of enterovirus infection in patients with heart diseases was proved to be considerably higher than that of other viruses. In a study in patients with MI, the high levels of antibodies against the enteroviruses, mycoplasma and chlamydia was associated with a higher risk of developing coronary heart disease, however, the study was conducted only in men without baseline history of heart diseases.[5] Particularly, infection with coxsackie B2 virus has been shown to have a great impact on heart disease (55.9%).[11] In another study, it was reported that the enteroviruses and herpes simplex viruses had the highest impact on the coronary artery disease.[14] Pesonen et al. performed a retrospective study to evaluate the role of viruses on heart diseases. They reported that the enteroviruses can greatly increase the risk of developing UA and acute MI.[15] In another study, the enteroviruses were reported in 49% of patients with ACS and 57.3% of patients with MI. This observation suggested that enterovirus infection is a risk factor for developing ACS and heart attack.[16] Evaluation of enterovirus infections and genetic polymorphism (at locus MMP3, MMP1, TNFα) among 208 patients with heart problems showed that 7% of blood samples were related to UA patients and 42% were related to those with MI. Prevalence of the enteroviruses in MI group were noticeably higher than UA group.[17] Our results are in accordance with the above findings and reinforce the hypothesis that enterovirus infections may play a role in the pathogenesis of UA.

Table 4. Detection of enteroviruses in patients with and without unstable angina according to the age groups

Age groups (year)	Enterovirus infection (RT-PCR)	With unstable angina (n = 51)	Without unstable angina (n = 55)	P
35-50	Positive	8 (66.7)	9 (52.9)	0.703*
	Negative	4 (33.3)	8 (47.1)	
51-65	Positive	7 (46.7)	5 (41.7)	0.795**
	Negative	8 (53.3)	7 (58.3)	
66-80	Positive	19 (79.2)	13 (50.0)	0.032**
	Negative	5 (20.8)	13 (50.0)	

* Fisher's exact test; ** Pearson chi-square test
RT-PCR: Reverse transcription polymerase chain reaction

In a study in Mashhad, only 2.6% of serum samples of patients with MI were positive for enterovirus RNA. This study did not find statistically significant results among different age groups or between men and women.[9] In contrast, we found a significant association between prevalence of enteroviruses infection and the incidence of UA in 66 to 80 years old patients. Furthermore, it was found that women with UA had a significantly higher prevalence of enteroviruses RNA in their blood samples compared to the control group. One explanation could be the fact that the other study was conducted in patients with acute MI.

The main advantage of our study was the use of PCR-based detection method which reveals the presence of the virus genome in blood samples of study subjects, instead of using serum antibodies which may show the past infection. The high prevalence of the enteroviruses could be due to the presence of various types of the enteroviruses.

In our work, the pattern of prevalence of the enteroviruses was not determined based on the genus and species and only the total prevalence was reported. Further investigations will be encouraged to categorize the enteroviruses and their prevalence in patients with UA. Besides, it would be of interest to gather more demographic information about the participants. Last but not the least, regarding the various prevalence of the enteroviruses in different seasons, further studies are encouraged to be performed in different time periods throughout the year.

Conclusion

Prevalence of the enteroviruses in patients with UA was significantly higher than those without UA in the elderly and women. There was no significant difference in other age groups and men. This study reports the link between enterovirus infection and UA but further studies with more power will be needed for better understanding of this relation.

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Conflict of Interests

Authors have no conflict of interests.

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