Survey of Coronaviruses Infection among Patients with Flu-like Symptoms in the Golestan Province, Iran

Hossein Khani (MSc)
Master of Science in Medical Virology, Student Research Committee, Golestan University of Medical Sciences, Gorgan, Iran

Alijan Tabarraei (PhD)
Assistant Professor in Medical Virology, Infectious Disease Research Center, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

Abdolvahab Moradi (PhD)
Professor in Medical Virology, Infectious Disease Research Center, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

Corresponding author: Abdolvahab Moradi

E-mail: abmoradi@gmail.com

Tel: +989111772107

Address: Golestan University of Medical Sciences, Gorgan, Iran

Received: 01 Oct 2017
Revised: 02 Nov 2017
Accepted: 22 Nov 2017

Abdolvahab Moradi
ORCID.org/0000-0003-8167-5469

ABSTRACT

Background and objectives: Coronaviruses are the main causes of respiratory tract infections in humans. They are also the second leading cause of common cold after rhinoviruses, and can lead to otitis media and asthma. The aim of this study was to investigate the molecular detection of coronaviruses in clinical samples of patients with flu-like symptoms.

Methods: Specimens were taken from 297 patients with flu-like symptoms who were referred to the influenza laboratory of Golestan University of Medical Sciences during 2012-2014. RNA was extracted from the specimens using an RNA extraction kit. Accordingly, RNA was used for cDNA synthesis and GAPDH was used as the internal control. Synthesized cDNA was investigated for presence of human coronaviruses genome with real-time polymerase chain reaction using specific primers. Data were analyzed by SPSS 16.0 software.

Results: The coronavirus genome was not detected in the specimens of patients with flu-like symptoms.

Conclusion: Genome of human coronaviruses is absent in samples from patients with upper respiratory tract infections and influenza-like symptoms, which may indicate the low prevalence of the virus in the Golestan Province, Iran.

KEYWORDS: Human coronaviruses, Upper respiratory tract infection, Golestan Province.

This paper should be cited as: Khani H, Tabarraei A, Moradi A[Survey of Coronaviruses Infection among Patients with Flu-like Symptoms in the Golestan Province, Iran]. mljgoums. 2018; 12(6):1-4

INTRODUCTION

Coronaviruses are positive stranded and enveloped RNA viruses belonging to the *Coronaviridae* family, which contain the largest genome among RNAs viruses (~27–33 Kb). Most human coronavirus infections are usually associated with mild upper respiratory tract infections accompanied with common cold-like symptoms (1, 2). Coronaviruses are divided into three serological groups, in which I and II mainly infect mammals (3,4). The coronaviruses identified as human coronaviruses include 229E, OC43, NL63, SARS, HKU1, and MERS.

Human coronaviruses HCoV-229E and HCoV-OC43 primarily cause self-limiting infections of the respiratory tract and usually induce mild to moderate common cold symptoms, such as rhinorrhea, headache, malaise, chills, sore throat, and cough (3). HCoV-NL63 has been estimated to be responsible for one-third of common cold-like illnesses in adults and severe pneumonia syndromes in young children (≤12 years old), the elderly, and immunocompromised patients (3, 5, 6). Human coronaviruses infections are mainly diagnosed from clinical respiratory tract specimens during winter and early spring with a reported frequency of 5-30%, which peaks in February (7). The SARS-CoV infection causes viral pneumonia with rapid respiratory deterioration, fever, chills, myalgia, malaise, and intestinal complications in adults and children (8, 9). The transmission routes of human coronaviruses are aerosols and fomite.

The severity of symptoms caused by human coronaviruses varies markedly among the infected individuals. The one-step real-time reverse transcription-polymerase chain reaction (RT-PCR) assay based on SYBR Green chemistry and degenerate primers targeting the conserved open reading frame 1b allow the detection of 32 animal coronaviruses including strains of canine coronavirus, feline coronavirus, transmissible gastroenteritis virus (TGEV), bovine coronavirus (BCoV), murine hepatitis virus (MHV), and infectious bronchitis virus (IBV). With a sensitivity of down to 10 RNA copies from TGEV, BCoV, SARS-CoV, and IBV, the assay can be considered a useful and sensitive technique for laboratory diagnosis and detection of still uncharacterized coronaviruses. In this study, we have utilized this molecular method for detection of human coronaviruses in specimens obtained from patients with flu-like symptoms.

MATERIAL AND METHODS

In this cross-sectional study, 297 throat specimens were obtained from patients referred to influenza laboratory of Golestan University of Medical Sciences, Gorgan, Iran between 2012 and 2014. All collected specimens were kept at -70 ^{o}C. Demographic data of patients were collected using a questionnaire.

Total RNA was extracted from the samples with an RNA extraction kit (Roche, Germany) according to the manufacturer's instructions. To confirm the integrity of the samples extraction, optical density of randomly extracted samples was evaluated. CDNA was synthesized according to the kit (Applied Biosystems, Lithuania) instructions. All cDNA synthesized were kept at -20 °C. RNA was incubated at 50 °C for 40 min for cDNA synthesis. GAPDH control was used to confirm cDNA synthesis.

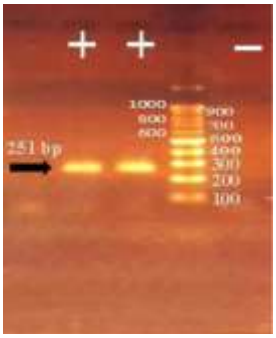
PCR was performed according to the method described by Vijgen et al. (10), Moës et al. (11), and Tatiane (12). Briefly, consensus primer pair encompassing the conserved region of coronavirus ORF1b (251 bp) was amplified. The following primer sequences were used in the PCR experiment: forward 5′-ACWCARHTVAAYYTNAARTAYGC-3′ and reverse 5′-TCRCAYTTDGGRTARTCCCA-3′.

PCR reaction solution (25 μL) contained 10X buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 100 pmol of each primer, and 1.5 unit/reaction Taq DNA polymerase. The amplification process started with activation of hot-start DNA polymerase at 95 °C for 15 min, and followed by 30 cycles of 94 °C for 30 sec, 48°C for 30 sec, and 72°C for 60 sec. PCR products were electrophoresed on 1.5% agarose gel stained with cyber green dye.

RESULTS

Among the 297 patients with flu-like symptoms, 75.1% were female (mean age: 37±15 years) and 24.9% were male (mean age: 34±21 years). The coronaviruse genome was not detected in the specimens of patients (Figure 1).

Figure 1- Gel electrophoresis of PCR product, positive control and negative control



Caught (85.9%) and sore throat (65.3%) were the most prevalent clinical symptoms, while obesity, pregnancy, AIDS, diabetes, vomiting, diarrhea, muscle pain and chronic heart disease, pulmonary disease, blood disorders, and liver disease were less frequent in these patients (>10%).

DISCUSSION

Respiratory viruses are a global health problem. Infections with the virus may be zoonotic and animals might play an important role in the virus transmission (13). Coronaviruses infect humans and many animal species.

These viruses may also cause otitis and asthma in humans. Most common clinical symptoms of coronaviruses strains HcoV-229E and HcoV-OC43 are headache, fatigue, diarrhea, sore throat, and caught. Rapid diagnosis of the viral respiratory infections notably affects clinical management and prevention of complications.

In this study, we showed no evidence of coronavirus genome in the specimens from patients with flu-like symptoms in the Golestan Province, Iran. We also showed that coronaviruses might not be an etiological factor for respiratory infections in this area. In studies conducted by Soltan i et al. (14) and Madahi et al. (15), the HcoV genome was found in 0.58% of specimens from male patients and in 5.5% of specimens from patients with respiratory symptoms, respectively. In the United States, human coronavirus was negative in 44 specimens collected during 2004-2005 from patients with respiratory illnesses (16). Several studies in different countries reported presence of human coronavirus in patients with respiratory illnesses (17-14). Absence of HcoV genome in clinical samples of respiratory tract infections may implicate low prevalence of the virus in the Golestan Province. Development of pancoronavirus multiplex PCR can provide new epidemiological and clinical aspects of the diseases caused by these viruses. Climate variations and other environmental factors may influence distribution of the virus. However, further serological analysis would help clarify the prevalence of coronaviruses.

CONCLUSION

Genome of human coronaviruses is absent in samples from patients with upper respiratory tract infections and influenza-like symptoms in the Golestan Province, Iran.

ACKNOWLEDGMENTS

This study was supported by the School of Medicine, Golestan University of Medical Sciences, Iran (Grant number: 941022235).

CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.

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