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Research Article

Prevalence of WU and KI Polyomaviruses in Negative SARS-CoV-2 Patients with Respiratory Symptoms in Hamadan, Iran

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Abstract

Objective: Respiratory infections are the most common disease worldwide. Recently studies have shown that viruses are the most important cause of respiratory infections. WU and KI Polyomaviruses (WUPyV and KIPyV) have a potential pathogenic role in respiratory infection. The global prevalence of WUPyV and KIPyV are between 1% to 16.4% and 0.45% to 12.14% respectively. The aim of this study is the prevalence of WUPyV and KIPyV among SARS-CoV-2 suspected patients with negative Real-Time RT-PCR tests.

Materials and methods: In this study nasopharyngeal swab samples were collected. Viral nucleic acid was extracted by High Pure Viral Nucleic Acid Kit (Roche, Germany) and Real-Time RT-PCR has been applied to detection of SARS-CoV-2. 602 negative samples were collected and PCR reaction was prepared by homemade primer to WUPyV and KIPyV. In this study DNA fragment of the virus was synthesized and cloned in the PUC57 vector as a positive control. Analytical sensitivity was determined by conversion of ng of plasmid DNA to copy number.

Results: Descriptive statistical analysis has been used by Graphpad prism V.8. In this study the prevalence of men and women were 53% and 47% respectively. The children under 5 years old were 3.8% of the total population. Elderly patients with more than 60 years of age were 35% of the study population. Analytical sensitivity of the PCR reaction was determined about 80 copies/reaction. WUPyV and KIPyV were not detected in these samples.

Conclusion: The prevalence of WUPyV and KIPyV are very different in the human population. These viruses are not found in Hamadan province. However, it seems that the other viral respiratory non WUPyV and KIPyVmay be considered in respiratory infections that were admitted with suspected COVID-19 symptoms.

Keywords: WU and KI polyomaviruses; SARS-CoV-2; Respiratory infections; Polymerase chain reaction

Introduction

Globally, respiratory viral infections cause significant mortality, especially in children. Respiratory viruses that are well known in human populations include Influenza viruses, parainfluenza viruses, Rhinoviruses, respiratory syncytial viruses, adenoviruses, human Coronaviruses, human bocaviruses, and human metapneumoviruses. WU and KI polyomaviruses have been identified in the respiratory secretions of human patients with acute respiratory infections [1-3].

WU (Washington University) and KI (Karolinska Institutet) viruses are in the family polyomaviridae that are small, double-stranded DNA, icosahedral, non-enveloped virus [4]. They were discovered in a respiratory sample of children and adults in 2007 and questioned their role in Respiratory Tract Infections (RTIs). There is disagreement as to whether WU and KI Polyomaviruses (WUPyV and KIPyV) are true respiratory pathogens. Since their initial discovery, they have been identified in respiratory specimens worldwide [5]. However, WUPyV and KIPyV were identified with other respiratory viruses [6]. In addition, a previous study reported that there is no evidence for a causal link between WUPyV or KIPyV infections and respiratory diseases [7].

Clinical manifestations in individuals with positive samples of WUPyV or KIPyV appear to be similar to those of other respiratory viruses and include fever (>38°c), cough, and other upper and lower respiratory symptoms [8-10]. Moreover, WUPyV has been found in the respiratory tract of patients without symptoms. Studies have identified viral sequences with similar or higher frequencies in asymptomatic patients, but few studies for KIPyV [10-12]. This study aimed to determine the prevalence of WU and KI Polyomavirus among SARS-CoV-2 suspected patients with negative PCR tests in Hamadan province, Iran.

Materials and Methods

Test specimens

At the Molecular and Genetic Medical Research Center, Vice Chancellor for Research and Technology, Department of Virology, School of Medicine, Hamadan University of Medical Sciences, nasopharyngeal swab samples were collected from outpatients and hospitalized (age range 1 day to 95 years) with respiratory symptoms from May 2021 to August 2021.

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Nasopharyngeal samples were collected in 3 ml - 4 ml of transport medium with antibiotics (Penicillin G 2 × 10^6 U/l and Streptomycin 200 mg/l) and kept at -70°C until testing [13]. The patients' clinical manifestations were fever (>38°C), cough, and shortness of breath.

Nucleic acid extraction

Nucleic acid was extracted from 200 µl of the sample using a High Pure Viral Nucleic acid extraction kit (Roche, Germany) for both viral RNA (SARS-CoV-2) and DNA (WUPyV, KIPyV) according to the manufacturer's instructions [14].

Detection of SARS-CoV-2

Detection of SARS-CoV-2 was performed by Real-Time RT-PCR, Nucleic Acid Diagnostic kit (PCR-Fluorescence Probing). The primer/probe is set for the *ORF1ab gene* and the *N gene* sequence for Real-Time RT-PCR. Detection limits were approximately 200 copies/ml for Real-Time RT-PCR.

Real-Time RT-PCR amplifications were performed in 50 µl reaction mixtures under the following conditions: 30 µl 2019-nCoV-PCR Master Mix (26 µl 2019-nCoV-PCR Mix+4 µl 2019-nCoV-PCR-Enzyme Mix) per the well, 20 µl of RNA extracted into the well pre-filled with reagent mixture, is as follows: Negative control 2019-nCoV-PCR, patient specimen(s), and positive control 2019-nCoV-PCR. At 2000 rpm, it was centrifuged for 10 seconds and placed in an ABI 7500 Real-Time thermocycler (Applied Biosystems, Foster City, CA). Real-Time RT-PCR amplification (30 minutes at 50°C for 1 cycle, 1 minute at 95°C for 1 cycle, followed by 45 cycles of 95°C for 15 seconds and 60°C for 31 seconds, 25°C for 10 sec, 1 cycle) was performed [15]. 602 negative SARS-CoV-2 samples were collected for WUPyV and KIPyV detection.

Plasmid colon

Plasmids containing the polyomavirus genome were used for positive control and to determine the assay's sensitivity by limit dilution analysis. Plasmid DNA was purified by a High Purity Plasmid Isolation kit (Roche Diagnostics). The concentration of purified plasmid DNA was determined with 200 nm - 1100 nm NanoDrop (Nabi, Microdigital, Korea). Based on the concentration and molecular weight of the plasmid, the corresponding DNA copies were calculated. A 10-fold dilution series of 10 copies/ml-3 copies/ml to 10 copies/ml-9 copies/ml of DNA polyomavirus was prepared for WUPyV and KIPyV in H_2O by PCR-grade, partially partitioned, and stored at -20°C.

Detection limit of WU and KI polyomavirus assay

To determine the sensitivity of assays, we prepared serial dilutions 10-fold of the DNA standard. The detection limit was defined as dilution containing the fewest copies of the viral genome, which still provides a positive result.

Detection of WU and KI polyomaviruses

The complete genome sequence was aligned by Clustal W in the MEGA program. Diversity sequences were found in the genome, and two conserved sequences were identified among the variable sequences. DNA primers for WUPyV and KIPyV that target the conserved region between VP1-VP2 were designed by SnapGene™ and compared by the Basic Local Alignment Search Tool (BLAST). GeneBank accession numbers were used in this study, EF444549 and EF127906 for WUPyV and KIPyV, respectively. KIWUF 5-TACCACTGTCAGAAGAAACAG-3 and KIWUSR 5-TGTTTCCATTCTGTACAGCTCCC-3 amplification primers were used for PCR, 250 bp and 290 bp amplitude lengths for WUPyV and KIPyV.

PCR amplification was performed in a 20 µl reaction mixture under the following conditions: 0.5 µl (0.25 µM) of each primer, 8 µl of nuclease-free water, 10 µl (1X) of MasterMix and 1 µl of DNA extract. This study used amplified VP1-VP2 fragments from WU and KI in the cloning vector (PUC57) as positive controls (double-stranded synthetic oligonucleotide dilutions from 4 × 107 copies/ml to 4 × 101 copies/ml) and ddH_2O as a negative control. Reactions were performed on the Biorad T100 thermocycler using a step cycle program. After the DNA internalization step at 94°C for five minutes, 35 cycles were performed: 94°C for 30 seconds, the annealing temperature of 56°C for 30 seconds and 72°C for 30 seconds with 10 minutes at 72°C extended after 35 cycles. 10 µl of each reaction was applied to 2% agarose gel (FMC Bioproducts, Rockland, Maine, USA) in TAE 1X buffer (0.04 mol/l Tris acetate, 0.001 mol /l EDTA) then safe stain (0.6 µL) to visualize PCR products amplified under UV light.

Results

Standardization of PCR

We applied a new PCR to detect WUPyV and KIPyV genomes in one reaction. The primers are designed with the NCBI BLAST program that selects protected regions for WUPyV and KIPyV isolates. We performed a homology analysis on the NCBI database to rule out possible interactions with unrelated viral sequences. No significant homology was found to indicate that these oligonucleotides designed in the region between the VP1 and VP2 sequences of both viruses could be considered for WUPyV and KIPyV amplifications. The sensitivity of PCR was determined by serial dilutions (from 4 × 107 copies/ml to 4 × 101 copies/ml) of synthetic dsDNA that carried specific sequences in the region between VP1 and VP2 of WUPyV (250nt) or KIPyV (290nt). Analytical sensitivities in singleplex form varied from 10 copies/ml-3 copies/ml to 10 copies/ml-9 copies/ml. The detection limit was dilution with the lowest copies of the viral genome, the fourth dilution (4 × 104 copies/ml). The detection limit in this study was approximately 80 copies/reaction for PCR.

Detection of WUPyV and KIPyV

In order to understand the epidemiological features of HPyVs in patients without SARS-CoV-2 in Hamadan province, 602 nasopharyngeal swabs from 45.8% outpatient and 54.2% hospitalized patients with respiratory symptoms were tested for the presence of WUPyV and KIPyV DNA. These patients ranged in age from 1 day to 95 years. Fifty-three percent of samples were from males, forty-seven percent were from females, 3.8% were collected from children 5 years of age or younger, and 35% were from patients older than 60 years. However, no positive samples were identified.

Discussion

As a result of this study, we determined the prevalence of WUPyV and KIPyV as respiratory pathogens in negative SARS-CoV-2 patients in Hamadan province, Iran, during the pandemic. We investigated the patients as the population of negative PCR cases, may include a higher proportion of other respiratory viruses.

WUPyV and KIPyV were not detected in any of the 602 nasopharyngeal specimens (319 (53%) males and 283 (47%) females), which is inconsistent with previous researches. Ligozzi et al. [16], in Italy reported that out of 482 patients, KIPyV was diagnosed at 3.1% and WUPyV at 4.9%, and two viruses were found in three samples of

simultaneous infection. In a study in Germany, out of 229 children with acute respiratory symptoms, KIPyV was 0.9% and WUPyV 0.4% positive [17]. In another study in Australia by Bialasiewicz et al. [18] of the 2866 different samples, 4.5% were positive for WUPyV and 2.6% were positive for KIPyV, ranging in age from 3 days to 95 years. Abed et al. [11] in 157 nasopharyngeal aspiration samples showed that of the children with respiratory symptoms, 2 (2.5%) of the 79 samples collected were WUPyV positive with a mean age of 13 months. Also, 5 of the 78 samples (6.4%) collected from asymptomatic cases were WUPyV positive with a mean age of 20 months. Rao et al. [19] in the Philippines found that among 1,077 nasal specimens from patients 6 weeks to 5 years of age with advanced lower respiratory tract infection, the prevalence and rates of co-infection were 5.3% and 74% for WUPyV and 4.2% and 84% for KIPyV, respectively. The difference in prevalence described in other studies may be related to changes in regional prevalence or age population selection, or nucleic acid amplification tests.

Preliminary studies have led to the hypothesis that WU and KI may play a role in respiratory infections, especially in immunocompromised children and adults [19]. The age distribution of patients with WUPyV and KIPyV showed two peaks, with the highest rates in children under 5 years and over 45 years [20,21]. Age distribution of our study showed 3.8%, <5 years old and 35%, >60 years old patient.

In this paper, in the first set of experiments, we set up a PCR to simultaneously determine WUPyV and KIPyV. This method was applied on respiratory samples to check the presence of those viruses in outpatient and hospitalized patients with respiratory symptoms that Real-Time PCR results for SARS-CoV-2 were negative. Our method detects WUPyV and KIPyV samples at 4 × 104 copies/ml, approximately 80 copies/reaction for PCR. In a similar study, the sensitivity of WUPyV PCR was 8.8 copies/reaction, which corresponded to 440 copies/ml of sample, and detected 4.9% WUPyV [21]. On the one hand, it may be due to the relatively low sensitivity of our method, but on the other hand, it may be due to the lack of WUPyV and KIPyV in the group of patients in Hamadan. Nevertheless, helpful equipment for WUPyV and KIPyV genome detection seems to be considered.

These two viruses showed very high co-infection rates with other respiratory viruses, which increased the complexity of WUPyV and KIPyV maps in respiratory pathogenesis. In particular, previous studies have shown that the rate of simultaneous detection of KIPyV with other respiratory viruses is about 74%, while WUPyV has shown rates ranging from 68% to 97% [8,10-12,19].

However, it is still controversial whether WUPyV and KIPyV are respiratory pathogens in the Iranian population. Aghamirmohammadi et al. [22] in Tehran province reported that of 206 children under 5 years old, WUPyV 1.5% and KIPyV 2% were detected, that is difference from our study. It would be because of geographical distribution. Further studies with more samples are needed to estimate the co-infection of WUPyV and KIPyV with other respiratory viruses in Hamadan province.

References

1. Mulholland K. Global burden of acute respiratory infections in children: implications for interventions. Pediatr Pulmonol. 2003;36(6):469-74.
2. Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, et al. Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog. 2007;3(5):e64.
3. Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, et al. Identification of a third human polyomavirus. J Virol. 2007;81(8):4130-6.
4. Johne R, Buck CB, Allander T, Atwood WJ, Garcea RL, Imperiale MJ, et al. Taxonomical developments in the family Polyomaviridae. Arch Virol. 2011;156(9):1627-34.
5. Rao S, Lucero MG, Nohynek H, Tallo V, Lupisan SP, Garcea RL, et al. WU and KI polyomavirus infections in Filipino children with lower respiratory tract disease. J Clin Virol. 2016;82:112-8.
6. Payungporn S, Chieochansin T, Thongmee C, Samransamruajkit R, Theamboolers A, Poovorawan Y. Prevalence and molecular characterization of WU/KI polyomaviruses isolated from pediatric patients with respiratory disease in Thailand. Virus Res. 2008;135(2):230-6.
7. Norja P, Ubillos I, Templeton K, Simmonds P. No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease. J Clin Virol. 2007;40(4):307-11.
8. Le BM, Demertzis LM, Wu G, Tibbets RJ, Buller R, Arens MQ, et al. Clinical and epidemiologic characterization of WU polyomavirus infection, St. Louis, Missouri. Emerg Infect Dis. 2007;13(12):1936-8.
9. Sloots TP, Whiley DM, Lambert SB, Nissen MD. Emerging respiratory agents: New viruses for old diseases? J Clin Virol. 2008;42(3):233-43.
10. Bialasiewicz S, Whiley DM, Lambert SB, Wang D, Nissen MD, Sloots TP. A newly reported human polyomavirus, KI virus, is present in the respiratory tract of Australian children. J Clin Virol. 2007;40(1):15-8.
11. Abed Y, Wang D, Boivin G. WU Polyomavirus in Children, Canada. Emerg Infect Dis. 2007;13(12):1939-41.
12. Han TH, Chung JY, Koo JW, Kim SW, Hwang ES. WU polyomavirus in children with acute lower respiratory tract infections, South Korea. Emerg Infect Dis. 2007;13(11):1766-8.
13. Dunn JJ, Pinsky BA. Specimen Collection, Transport, and Processing: Virology. Manual of Clinical Microbiol. ASM Press; 2015. p. 1405-21.
14. https://www.lifescience.roche.com
15. http://eng.sansure.com.cn/index.php?g=portal&m=article&a=index&id=81
16. Ligozzi M, Galia L, Carelli M, Piccaluga PP, Diani E, Gibellini D. Duplex real-time polymerase chain reaction assay for the detection of human KIPyV and WUPyV in nasopharyngeal aspirate pediatric samples. Mol Cell Probes. 2018;40:13-8.
17. Mueller A, Simon A, Gillen J, Schildgen V, Tillmann RL, Reiter K, et al. Polyomaviruses KI and WU in children with respiratory tract infection. Arch Virol. 2009;154(10):1605-8.
18. Bialasiewicz S, Whiley DM, Lambert SB, Jacob K, Bletchly C, Wang D, et al. Presence of the newly discovered human polyomaviruses KI and WU in Australian patients with acute respiratory tract infection. J Clin Virol. 2008;41(2):63-8.
19. Rao S, Lucero MG, Nohynek H, Tallo V, Lupisan SP, Garcea RL, et al. WU and KI polyomavirus infections in Filipino children with lower respiratory tract disease. J Clin Virol. 2016;82:112-8.
20. Abedi Kiasari B, Vallely PJ, Corless CE, Al-Hammadi M, Klapper PE. Age-related pattern of KI and WU polyomavirus infection. J Clin Virol. 2008;43(1):123-5.
21. Neske F, Blessing K, Ullrich F, Pröttel A, Wolfgang Kreth H, Weissbrich B. WU polyomavirus infection in children, Germany. Emerg Infect Dis. 2008;14(4):680-1.
22. Aghamirmohammadali FS, Sadeghi K, Shafiei-Jandaghi NZ, Khoban Z, Mokhtari-Azad T, Yavarian J. Survey of WU and KI polyomaviruses, coronaviruses, respiratory syncytial virus and parechovirus in children under 5 years of age in Tehran, Iran. Iran J Microbiol. 2020;12(2):164-9.