Iranian Journal of Virology 2023;17(1):15-25.
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Original Article

Detection of Respiratory Viruses in Children Less than 5 Years of Age with Suspected COVID-19 Infection in East Azerbaijan Iran

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Abstract

Background and Aims: Coronaviruses are one of the most common viral agents associated with respiratory disease in children, causing severe respiratory infections and hospitalizations in children. However, there is no information about the prevalence of these seasonal coronavirus infection in the northwest of Iran. The aim of this study was to identify and investigate the frequency of NL63, OC-43 and 229E, Influenza type A, B viruses in children less than 5 years of age with respiratory infection in the northwest of Iran during the COVID-19 pandemic.

Material& Method: In the present study, 164 respiratory samples were taken from children with respiratory problems who were negative for influenza type A, B and SARS-CoV-2 (Taqman Real Time RT-PCR method). NL63, OC-43 and 229E viruses were evaluated in a panel by Cyber green RT-PCR method.

Results: In our investigation, 2 out of 164 (1.2%) cases of NL63 infection were detected in children less than one year, but OC-43 and 229E viruses were not detected. NL63 positive samples in this study were detected in late winter and early spring.

Conclusion: Considering the role of this virus in causing respiratory problems in children and immunocompromised patients, identification and diagnosis of these pathogens in respiratory samples is important. Paying attention to viral infections in the northwest of Iran can play an important role in improving the management of infectious agents in this area.

Keywords: Coronaviruses, SARS-CoV-2, HCoV-NL-63, HCoV-OC-43, HCoV-229E, Acute Respiratory Tract Infections (ARTI)

Introduction

Coronaviridae is a large family of viruses with a virions size of 120 to 160 nm that has an RNA genome (Monopartite, linear ssRNA(+) genome of 27-32kb in size the largest of all RNA virus genomes, 5'Capped, and 3' polyadenylated) (1). The term "coronavirus" originates from the appearance of the virus with crown-like protrusions caused by spikes on the surface of the membrane (S glycoproteins) when viewed with an electron microscopy (2). Based on serological and genetic differences, human coronaviruses are divided into three separate groups (3).

Coronaviruses are known to cause respiratory and digestive diseases in a wide range of mammals and birds, including pigs, dogs, cats, horses, chickens, turkeys, rabbits, cattle and humans (4).

From the beginning of the identification of the first coronaviruses in the early 1960s, the most

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important focus and importance was on investigating the pathological conditions of coronaviruses in domestic animals, and human. In the middle of the 1960s, epidemiological studies led to the identification of Human Coronavirus-229E (HCoV-229E) and Human Coronavirus-OC43 (HCoV-OC43) -(5). The identification of another Human Coronavirus-NL63 (HCoV-NL63) virus in winter 2002 to 2003 in a 7-month-old child with symptoms of bronchitis and conjunctivitis, followed by the identification of this virus in several other children and adults, increased the importance of coronaviruses in causing respiratory diseases. Studies have shown that all age groups are susceptible to mild respiratory diseases and severe involvement of the lower respiratory tract in infants and the elderly with coronaviruses, and even re-infection with these viruses is possible (6).

Viruses are among the most common causes of ARTI in children, especially in infants and preschool children. The most common respiratory viruses in children are influenza A and B (IFV), parainfluenza viruses (PIV), human respiratory syncytial virus (HRSV), rhinoviruses (RV), adenoviruses (ADV), metapenoviruses, and bocaviruses. and human coronaviruses (HCoV) (7).

Human coronaviruses (HCoV) OC43, 229E, NL63, and HKU1 are associated with upper and lower respiratory tract infections. Despite the global distribution of coronaviruses, it is possible that the dominant species have different distribution depending on the region or year (8, 9). Coronaviruses, such as NL63, can cause flu-like symptoms, which makes it difficult for children to be properly diagnosed and even treated. In some cases, this issue causes pneumonia and respiratory distress in children, which eventually lead to hospitalization in the intensive care unit and even lead to death (10-12). Among the identified coronaviruses, SARS-CoV-1 with the outbreak of severe respiratory disease during 2002-2003 in China and MERS-CoV as the cause of the outbreak of severe respiratory diseases in the Middle East in 2012 as acute respiratory diseases with involvement of the lower part of the respiratory tract were known and have the highest death rate among coronaviruses (13, 14).

OC43, 229E, NL63 viruses are well documented in the studies conducted in Iran. However, we do not have accurate and sufficient information about the prevalence and circulation of these viruses in the northwest of Iran. The aim of the present study is to identify these viruses in the population of children fewer than 5 years of age as a population at risk for these viruses. We also investigate the seasonal circulation of these viruses in different months of the year in the northwestern of Iran.

Methods and Materials

Characteristics of Patients

Children less than 5 years of age, who had been referred to the children's hospital in Tabriz during the winter 2020 with complaints of respiratory symptoms were included. Most patients had mild respiratory symptoms, and some were admitted with severe respiratory symptoms. Nasopharynx and oropharynx samples were taken from the all patients. Initially, all patients were checked for infection with influenza virus and SARS-CoV-2.

Afterwards, 164 samples from patients (children under 5 years of age) with negative results for influenza and SARS CO2 infection were collected and stored at -80°C until the examination of seasonal coronaviruses.

Inclusion Criteria

Children under 5 years of age with respiratory symptoms referred to the Tabriz Children's Hospital.

RNA Extraction and Reverse Transcription

RNA was extracted using the RNJia Virus kit (Rojetechnologies, Iran) as instructed by the manufacturer. 140 µl of sample was used for RNA extraction. Extracted RNA was eluted in 50 µL of DEPC water and stored at −80 °C.

Complementary DNA was amplified in the ABI Prism 7000 Sequence Detection System (ABI, Singapore), cDNA was produced by First Strand cDNA Synthesis Kit (SinaClone, Iran), cDNA was used to detect other coronaviruses. The quality of all extracted RNA was

evaluated using Primers and probes specific to the Rnase P gene were used (Table 1). Rnase P negative samples were re-extracted. Sterile distilled water was used as a negative control.

Detection of Influenza A and B Viruses and SARS-CoV-2

All Rnase P positive samples were evaluated separately by specific primers and probes for the diagnosis of influenza A and B (Table 1), NL63, 229E, and OC43 coronaviruses (Table 2). Detection of the SARS-CoV-2 was performed using the commercial RT-PCR Covid-19 single-stage kite kit according to the protocol recommended by the manufacturer (PCR-RT Step-One 19- Pishtaztebzaman, Iran). This kit targets and identifies the SARS-CoV-2 RDRP and N genes. Positive and negative samples were used at each stage to evaluate the results (Table 1).

Detection of Common Coronaviruse

NL63, 229E, and OC43 coronaviruses were detected according to the method proposed by Sultani et al, (15) by using multiplex SYBR Green real-time PCR (15).

According to this method, a 10-minute step at 94°C was first defined for initial denaturation. This was followed by 40 cycles with 15-second conditions at 95°C for denaturation, one minute at 55°C for annealing, and one minute at 60°C for amplification of the target gene and fluorescent reception. The specificity of PCR products was determined by melting curve. At the end of the 40 cycles, the temperature rose to 94°C for 3 minutes, then reduced the temperature to 65°C and increased it alternately at a rate of 0.1°C per second to reach 94°C. The fluorescent receiving process was recorded with increasing temperature and the melting curve was obtained by the software (Table 2).

Statistical Analysis

Statistical analysis was carried out using SPSS (Chicago, IL, USA) software version 23 for Microsoft Windows®. The results were processed statistically using the Chi square test and T-test.

Results

In the present study, during a one-year period of 2020-2021, a total of 164 samples of children were collected in different months of the year. Children in this study were 74 girls (45.13%) and 90 boys (54.87%) with the average age 23.4 months. 25.60% (n=42), 45.13% (n=74) and 29.27% (n=48) were under one year, one to three years and three to five years, respectively. No significant difference was observed between the data in terms of gender (P value= 0.315).

First, the RNA extraction of all samples was evaluated by identifying the Ribonuclease P gene (Rnase p - internal control) using primers and probes specific to this human gene (Table 1). All samples were positive in terms of internal control (Figure 1).

All samples were tested for influenza A and B and SARS-CoV-2 by the Taqman Real Time RT-PCR method. All samples were negative for these viruses (Figure 2).

Based on the size of the final PCR product and the specific sequence amplified during the PCR process, if there was contamination, the specific melting temperature for each of the NL63, OC43, and 229E coronaviruses will be obtained. In the current study, out of three seasonal coronaviruses NL63, OC43, and 229E, only 2/164 (1.2%) patients with NL63 infection were identified (Figure 3).

In terms of clinical characteristics, the most common symptoms in this study were fever, mild cough, and runny nose. Both patients identified with NL63 infection were very similar in terms of age and clinical symptoms. However, there were tangible differences in the clinical characteristics of both patients (Table 3).

Discussion

The present study, was done to determine HCoV-NL63 virus infection for the first time in the northwest of Iran. In 1965, the first human coronavirus (strain B814) was isolated from a respiratory patient with cold symptoms. Subsequently, many strains of this family have been identified. Among the human coronaviruses, HCoV-229E was identified from a student using standard tissue culture (229E:

Table 1. Primers & probes for detection: RnaseP and Influenza (Type A&B)

Gene target	Name	Sequence (5'-3')	Product size(bp)	Ref.
Rnase P	Forward	AGATTTGGACCTGCGAGCG	65	50
	Reverse	GAGCGGCTGTCTCCACAAGT		
	Probe	FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ1		
Influenza Type A (M)	Forward	GACCRATCCTGTCACCTCTGAC	106	
	Reverse	AGGGCATTYTGGACAAAKCGTCTA		
	Probe1	FAM-TGCAGTCCTCGCTCACTGGGCACG-BHQ1		
Influenza Type B (HA)	Forward	AAATACGGTGGATTAAACAAAAGCAA	170	
	Reverse	CCAGCAATAGCTCCGAAGAAA		
	Probe1	Fam-CACCCATATTGGGCAATTTCCTATGGC-BHQ1		

Abbr: Rnase P, Ribonuclease P; BHQ1, Black Hole Quencher-1; M2, Matrix Protein2; HA, Hemagglutinin

Table 2. Primers for detection HCoVs (OC43, NL63, 229E).

Virus (Target gene)	Sequence (5'-3')		product size (bp)	Tm	Ref.
OC43 (S)	Forward	CGATGAGGCTATTCCGACTAGGT	76	89.26	15
	Reverse	CCTTCCTGAGCCTTCAATATAGTAACC			
NL63 (ORF1b)	Forward	ACGTACTTCTATTATGAAGCATGATATTAA	103	81.4	
	Reverse	AGCAGATCTAATGTTATACTTAAAACTACG			
229E (M)	Forward	CATACTATCAACCCATTCAACAAG	137	94.47	
	Reverse	CACGGCAACTGTCATGTATT			

Abbr: Tm, temperature melting; S, spike; ORF1b, open reading frame 1b; M, Matrix.

Table 3. Clinical Features of Infants (n = 2) With Coronaviruses NL63

Clinical Features	Patient Number 1	Patient Number 2
Age (Month)	10	11
Sex	male	female
Season (month)	Winter (March)	Spring (April)
Admission to pediatric ICU	No	No
Cough	Yes	Yes
Fever (>38°C)	Yes	Yes
Runny nose	Yes	Yes
Conjunctivitis	No	Yes
Sleep disrupted	No	No
Restlessness	Yes	No
Daily activities Disrupted	Yes	No
Antibiotic Use	No	No
Symptoms duration (Weeks)	4	2
Diarrhea	No	No
Temporary loss of appetite	Yes	No

student sample code), and HCoV-OC43 (Organ Culture 43) from a tracheal organ culture. Its serological difference with 229E confirmed the identification of the new strain, also from the aspiration of a 7-month-old infant with bronchiolitis in the Netherlands NL63 (Netherlands 63) and from a Hong Kong patient with pneumonia HCoV-HKU1 (The University of Hong Kong 1) have been identified (6, 16).

Most reports of NL63 involvement have been reported from young children, the elderly and immunocompromised patients. After identifying this virus in a Dutch baby with symptoms of coryza, conjunctivitis, fever, and bronchiolitis, the same virus was isolated from 8 immunocompromised patients, including children and adults, in 2004. Later, in 2005, this virus was isolated from 79 children out of a total of 895 children with cold symptoms in

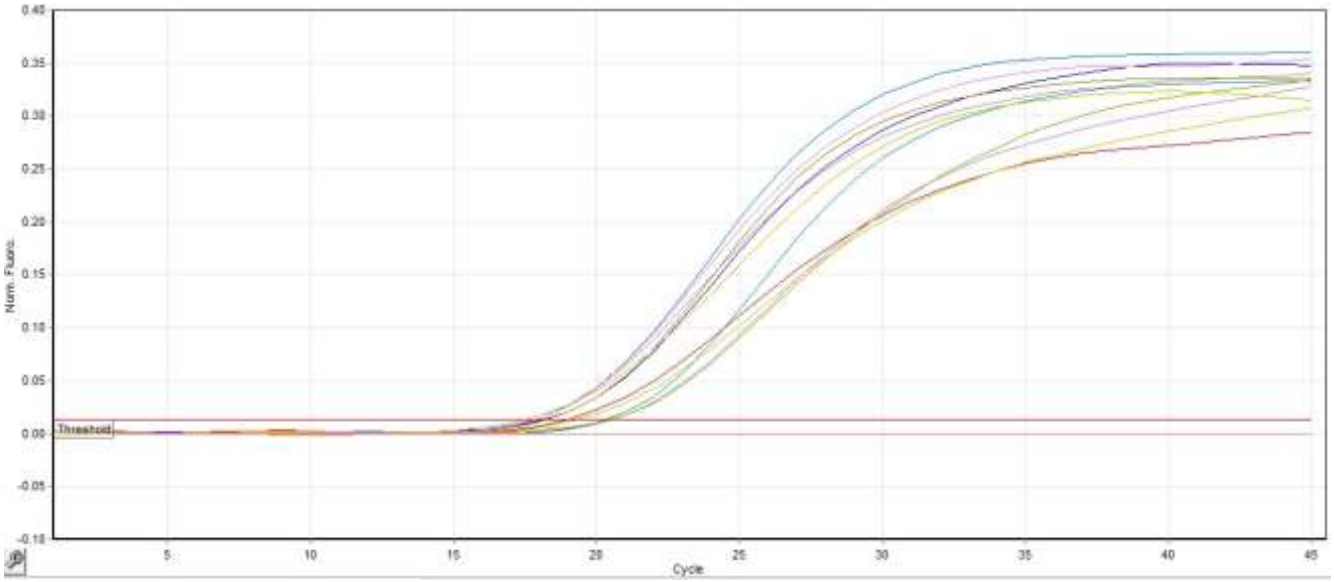


Fig 1. The amplification plot for some samples with Real Time Taqman RT-PCR method to identify the human RNase P gene (Internal Control). All samples were positive with CT 15-18; the curve is below the negative control sample's threshold line.

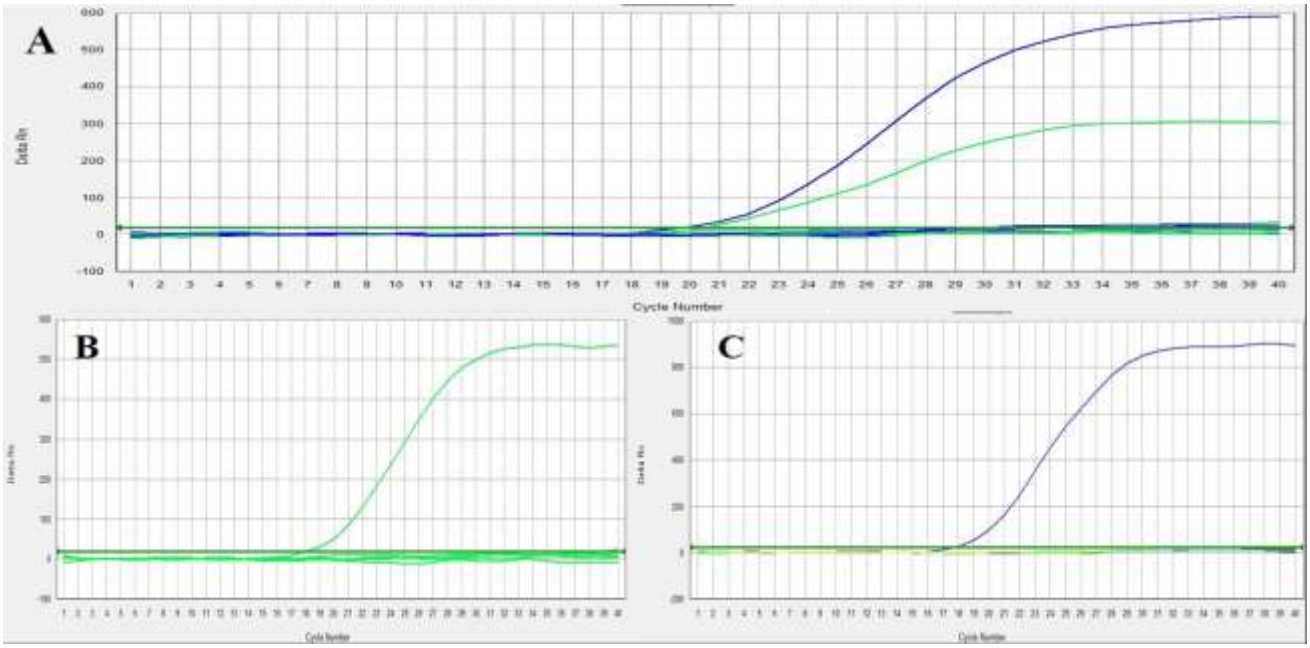


Fig 2. Amplification plots for nasopharynx-oropharynx samples by Real Time Taqman RT-PCR method for detection of influenza A virus, influenza B virus and SARS-CoV-2. All samples were negative for these viruses. (A) Linear curves for SARS-CoV-2 positive control (green curve: N gene, blue curve: RdRP gene) and some negative samples, (B) Linear curve for posi-tive control of influenza type A (green curve) and some negative samples for this virus, and (C) linear curve for the positive control of influenza type B (blue curve) and some negative samples for this virus.

New Haven, USA (14). In the current study, we identified 2 children with NL 63, aged 10 and 11 months old.

Different age distributions of patients have been reported among diverse HCoV strains. In other words, HCoV-229E is the most prevalent among adults over 18 years old, and HKU1, NL63, and -OC43 are the most reported among young children and infants (17, 18).

Nowadays, coronaviruses are one of the most important causes of respiratory tract infections in humans. These viruses have been observed with different pathogenic functions among age

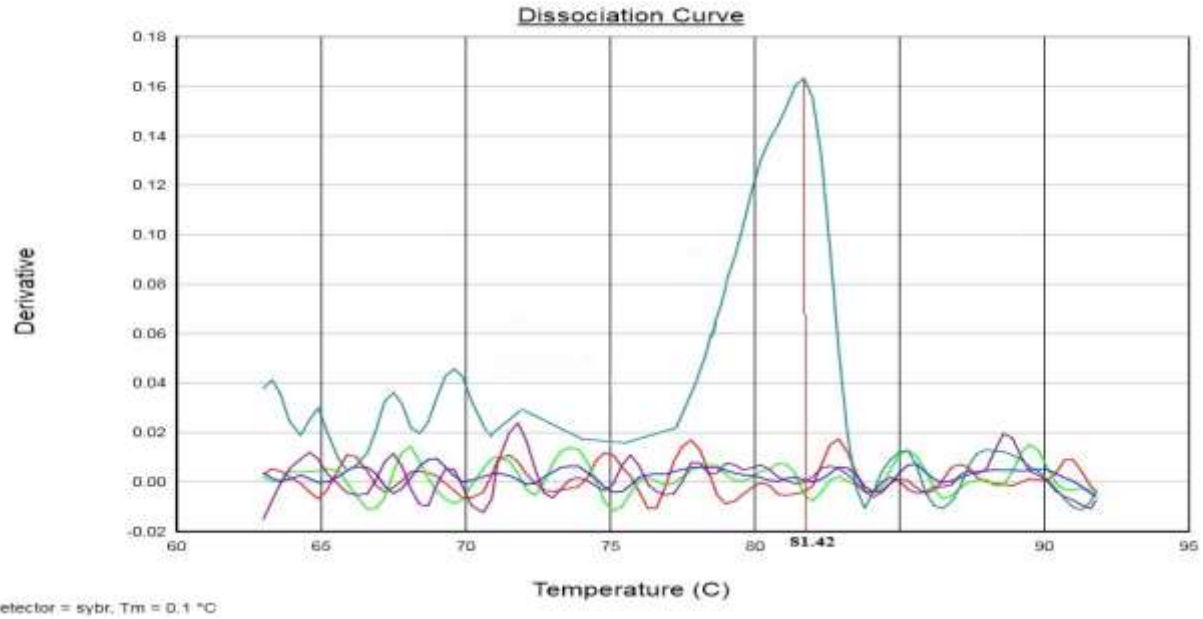


Fig 3. Real-Time PCR melting curve in a positive specimen for NL-63 virus. Melting curve for a positive sample of HCov-NL63 virus with cycle threshold of 27 (Ct 27) and (Tm 81.42) (turquoise color curve) and 4 negative samples for Human Coronaviruses NL63, OC43, and 229E viruses based on the Sybr-green Real Time RT-PCR method for nasopharynx-oropharynx samples.

groups. In common coronaviruses, HCoV-NL-63 is clinically more important among young children and infants (19).

Several studies have been conducted in the field of HCoV-NL-63 identification, which shows the diverse prevalence of this virus in countries. In a large study in Kenya conducted by Kiyuka et al., the prevalence of NL63 in 5573 nasal samples taken from children under 5 years of age was reported as 1.3% (20). However, in China, NL63 has been reported as an important respiratory pathogen in infants and the elderly in some studies. It has also been stated that this virus can cause severe respiratory disease in infants, young children, the elderly, and immunocompromised people (21). It has been shown that the virus maintains its infectious ability for up to seven days in aqueous solution and respiratory secretions, and can remain infectious for some time at room temperature. Furthermore, considering person-to-person transmission as the main route of NL63 spread can strengthen the spread of infection in densely populated and high-density areas (schools and kindergartens) and the difference in reporting the prevalence of this virus in different studies (21).

In a study in Japan in 2005 by Suzuki et al. Of the 419 samples that were negative for common respiratory viruses, 5 samples (1.2%) were reported to be positive for human coronavirus NL63 (22). However, in another study in Japan in 2015, of 118 nasopharyngeal swab samples from hospitalized children under two years of age, three samples (2.5%) were positive for HCoV-NL63 (23).

In Melbourne, Australia, in a study conducted on 543 patients with respiratory symptoms, 18 cases (3.3%) of the NL63 virus were identified and reported (24).

Several studies in the United States have also reported that one to 10% of the Washington population is infected with NL63 annually (25). Different outbreaks have been reported in European countries. In a study in Italy, 322 infants with acute respiratory disease, among which 21.4% were of HCoV-NL63 type (26). Also, in the study of Vabret et al., in France, 28 samples (9.3%) were positive out of 300 respiratory samples examined for NL63 detection (27).

The results of our study show that the NL63 virus has been circulating among hospitalized children in northeastern Iran in winter and spring (April-March). However, due to various reports of the spread of the virus in different seasons, there are uncertainties about the role of seasonal change in the identification of this

virus. In general, 229E, OC43, and NL63 are globally distributed, and transmission of these viruses is most commonly reported during winter in temperate countries. On the contrary, a summer-spring activity peak with NL63 outbreaks has been reported in a study in Hong Kong (28, 29).

In a research from Vabret et al., the highest prevalence of NL63 was expressed in the respiratory samples taken from children in February. In this study, NL63 was isolated in nasal aspiration every month during the study, however, the highest prevalence was related to winter (27). Despite the inconsistencies in the seasonal spread of coronaviruses, in most reports, the spread of the virus has been the most reported in winter and the months of January to March (6, 30, 31). In the present study, one of the two cases was identified in winter and one case in the early spring. In the northwest region of Iran, the air temperature is low in early spring, similar to winter. Despite these reports, the highest prevalence in China was mainly in spring and summer (29). There is conflicting information about the spread of corona and viruses in different seasons of the year, for example, In a study published on the spread of the coronavirus in Thailand, it was reported that there is no connection between the spread of the virus and seasonal changes, and that coronaviruses do not tend to a specific season (12), but in another study, Wu et al., in Taiwan, reported that coronaviruses, especially NL63, are more common in autumn (32).

These various studies show that the coronavirus has the least preference for a certain season, temperature changes do not have significant effects on its spread, and infection can occur throughout the year.

Similar to our study, other studies have been conducted with the aim of identifying coronaviruses associated with seasonal colds, NL63, OC43 and 229E in Iran, which have resulted the same outcome to our study. Nevertheless, compared to other parts of the world, few studies have been conducted in Iran in the field of identifying coronaviruses associated with cold symptoms. In a study by Madhi et al., in Tehran, among 270 samples of hospitalized patients due to various types of respiratory infections, 0.58% were positive for NL63 coronavirus (33).

There have been several reports about human coronas and other respiratory viruses in Iran. NL63 was reported for the first time in a study conducted in Iran in 2012 by Sultana et al. This virus was isolated from a 28-day-old girl among 322 respiratory samples (one case (0.58%) by using the multiplex SYBR Green-Time PCR method (15).

A recent study by Mohammadi et al. in Kerman province of Iran shows a relatively high prevalence of NL63 among children less than five years of age with respiratory symptoms. These results indicate the high prevalence of NL63 in this geographical location. Besides, in their study, the highest prevalence related to the age group of fewer than 12 months was reported (34).

Out of the two patients with NL63 infection in the present study, one patient with a history of traveling to Turkey ten years prior to sampling was recorded (a country with a common border from the northwest side of Iran). However, confirming the origin of viral infection requires cross-country phylogenetic studies. There are many reports of coronaviruses in Iran's neighboring countries. In Turkey, Kenka et al. have isolated and reported a case of NL63 coronavirus from seven-month-old premature babies (53) in the investigation of the causes of death of infants with lower respiratory disease (35).

However, in another study in Turkey by Agca et al., none of the other coronaviruses were reported during the first year of the SARS-CoV-2 epidemic (36). In the study of H. A. Salah et al. in Iraq from another neighbor of Iran (with a common border in the west and northwest of Iran) in 2020, there is also an outbreak of NL 63 and 229E viruses (37). However, the HCoV-NL63 virus has been detected in other countries around Iran. Al-Hajjar et al., Saudi Arabia in 2011 reported that among children with upper and lower respiratory diseases in the fall and winter of 2007–2008, 2.8% of samples were infected with NL63 (38).

Both patients identified in our study were hospitalized due to severe respiratory symptoms (fever and mild cough) (Table 2). In

addition to respiratory problems, some reports show that NL63 is one of the pathogens associated with Kawasaki disease (39), however, other studies do not confirm this connection (40, 41). Infection with NL63, with global distribution, generally causes a mild respiratory disease similar to a cold, the most common symptoms of which are cough, rhinorrhea, tachypnea, fever, and hypoxia (22).

In a study of 592 children less than six years of age admitted to the Children's Hospital of Ljubljana, the capital of Slovenia, the HCoV was detected in 6% of all samples. Of these samples, HKU1, OC43, 229E and NL63 were identified in 52.5%, 17.5%, 15% and 15%, respectively (42).

Even though SARS-CoV and HCoV-NL63 use a common receptor, angiotensin-converting enzyme (ACE)-2, to enter cells, however, unlike SARS-CoV, which causes a severe respiratory infection, NL63 is a mild, and it is self-limiting (62). Despite the use of a common receptor, NL63 has been shown to have a weaker interaction with ACE-2 than SARS-CoV, which could be the reason for the mild infection of this virus compared to severe respiratory infection following SARS-CoV (43).

In another recent study by Aghamirmohammadali and colleagues in Iran, a total of 16 people (7.76%) were HCoV. This study was conducted on samples collected from several different regions of Iran. Among HCoVs, they reported 7 (3.40%) 229E, 5 (2.42%) HKU1, 3 (1.45%) OC43, and 1 (0.49%) NL63 (44). In a large study in China, out of 11,399 samples of hospitalized children with respiratory problems, a total of 4.3% (11,399/489) were positive for HCoV. In their investigation, the highest prevalence was reported for OC43 (3%) and the lowest for HKU1 (0.3% (45).

Gaunt et al. (2010) in England analyzed 11,661 respiratory samples over 3 years between July 2006 and June 2009 for human coronaviruses using a new real-time four-way multiplex. In total, they detected the coronavirus in 0.3 to 0.85 percent of the samples in all age groups. The study reported that, except for HCoV-229E, which was detected year-round in 2008, other coronaviruses showed a seasonal pattern with a distinct winter outbreak from April to December and were not detected during the summer months (18).

Moreover, in another study by Zhang et al., in southern China, 13,048 samples of nasal secretions from patients of different ages (infants and adults) with respiratory tract problems was collected for 5 years (2010 to 2015). In 294 samples, one of the four coronaviruses OC43, 229E, NL63, and HKU was detected. Most infections with coronaviruses have been reported in age groups fewer than three years old and over 50 years old. Also, in this study, OC43 was the most prevalent (25). Dar et al. (2007)., 21 people (1.8%) out of 1156 patients with pneumonia, 12 people (2.3%) out of 513 outpatients, and 6 people (2.1%) out of 281 patients had HCoV infection, and the highest prevalence in this study It was also related to the OC43 virus (12).

In the study by Killerby et al. in England the years 2014 to 2017 in the investigation of human coronaviruses, it was reported that HCoV-OC43 was 2.2% more common than other species and this virus reaches its peak during the year, while HCoV NL-63, HCoV-HKU1, and HCoV-229E show greater variability, peaking within a few years (18).

Some studies have suggested that NL63 had an ancestor HCoV-229E in the past, and over time, it gave rise to two separate lineages of NL63. At present, it has been observed that these two lineages have been combined due to simultaneous infection and have finally formed two genotypic subgroups. Currently, a combination of NL63 clinical viruses has been observed circulating in the human population (46). Phylogenetic analyzes have shown that NL63 is closer to the first group of coronaviruses and belongs to this group. The highest similarity is observed with HCoV-229E and porcine epidemic diarrhea virus (PEDV), 65% and 61%, respectively (18).

Despite the lower prevalence of NL63 in studies compared to other human coronaviruses, this virus is associated with more severe respiratory symptoms and acute respiratory disease in children under 1 year of age and immunocompromised adults (6). Infection with 229E virus appears with symptoms such as general weakness, headache, nasal discharge,

sneezing, and sore throat. 10 to 20% of patients also have fever and cough, and clinically it cannot be distinguished from other respiratory tract infections such as rhinovirus and influenza (47, 48).

The 2019 coronavirus (COVID-19) pandemic has spread globally with millions of confirmed cases and more than 7 million deaths by the World Health Organization (WHO) (as of May 2021). The importance of this issue has caused many researchers to try to better understand and control this disease in the field of identifying pathogenesis, ways of spreading, ways of control, and treatment (49).

Conclusions

In the present study, the only strain identified was NL 63 and other strains were not identified. One of the reasons for this issue can be geographical differences in the spread of respiratory diseases related to coronaviruses or Lowe samples size. In the studies conducted in Iran, most reports were of the NL 63 strain; however, other strains have also been reported in Iran.

The importance of determining the annual circulation patterns and the correlation of the prevalence of HCoV infection with the change of season has become clearer than before. The existence of many types of Coronaviruses circulating in nature with wide host diversity has greatly increased the possibility of interaction between viruses of this family, which always leads to the possibility of the next recombinant Covid-19 emerging in the community and another circulation in the human population. However, the need for molecular techniques is felt due to the lack of assays to detect intracellular antigens or other serological tests and the difficulties in culturing the virus. Multiplex RT-PCR can be used as a useful and practical tool in the simultaneous detection of different HCoV from a sample taken from a patient. Continued use of multiple respiratory virus assay panels could facilitate further definition of circulating HCoV through public health surveillance in the future.

Acknowledgment

We appreciate from Dr. Professor, Shahram Abdoli Oskouee, Pediatric specialist Tabriz Children's Specialized Hospital for in the identification of Children with suspected respiratory symptoms and data gathering. I'd also like to acknowledge the team of staff of virology in the central laboratory of Tabriz province for supporting study equipment.

Authors' Contributions

The registration of people's information, sampling, sample processing, identification, examinations patient have been completed by Moslem Ghasemi Nia and patiently verified the accuracy of data analysis, study concept etc. was done by Mrs., M. Ahangar Oskouee co-supervised; and all the other authors honorably revised the final version of the paper, approved the manuscript and accepting accountability for all aspects of the work.

Disclosure

This study was extracted from a research project, "Detection of respiratory viral load in respiratory specimens of patients with suspected Covid-19 in East Azerbaijan province" presented by Mahin Ahangar Oskouee.

Data Availability

The data that support the findings of this study are available and included within the article.

Conflict of Interest

No conflict of interest is declared.

Funding

This study was supported by a grant from Tabriz University of Medical Sciences (Tbzmed) and ethical code is: IR.TBZMED.REC.1399.646.

Ethics Approval and Consent to Participate

Not applicable.

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