Alpha and Beta Coronavirus Infections in a University Hospital

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Authors' contributions

This work was carried out in collaboration among all authors. Author HA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IS and BE managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

Original Research Article

ABSTRACT

Background: Human coronaviruses (CoV) are significant causes etiological factors of respiratory tract infections. There are four major subgroups of CoVs, known as alpha, beta, gamma and delta. Four types of endemic human CoVs are 229E, OC43, NL63 and HKU1.
Aims and Objectives: In this retrospective study, we aimed to analyze the results of the respiratory samples of hospitalized patients by Real-Time(RT) PCR for CoVs 229E, NL63,OC43 and HKU1.
Methodology: Hospitalized patients with respiratory symptoms, including nasal congestion, rhinorrhea, nasal discharge, sneezing, sore throat and cough, were tested by RT PCR between April 2018 and March 2020. We retrospectively investigated the results of 1422 respiratory samples including 1245 nasopharyngeal swabs and 177 BAL samples. Detection rate and subtypes of CoV among upper and lower respiratory samples were retrospectively analyzed from patient records.

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Results: Patients were between 0-93 years with a mean age of 26 years. We detected 142 (11,4%) CoVs in 1245 nasopharyngeal samples and 21 CoVs (11,9%) in 177 BAL samples. Most common CoV was OC43 (n=72, 44,2%) in overall samples.
Conclusion: Although CoV infections are predominant in the winter season, they are diagnosed throughout the year with lower incidence in summer and are identified in individuals of all ages. Understanding the epidemiological and virological features of CoV infections is important to effectively control their burden.

1. INTRODUCTION

Respiratory tract infections are common all around the world and are associated with major health burden [1,2]. Human coronaviruses (CoV) are significant causes of respiratory tract infections. CoV is an enveloped RNA virus with positive polarity and a single chain. There are four major subgroups known as alpha, beta, gamma and delta while the beta coronaviruses are further divided into A,B,C,D lineages. Four types of endemic CoVs are 229E, OC43, NL63 and HKU1. Coronaviruses are zoonotic viruses [3-5]. Alpha coronaviruses are 229E, NL63 and beta are HKU1 and OC43 in the lineage A, Severe Acute Respiratory Syndrome (SARS) CoV and SARS-CoV-2 are in the lineage B and MERS CoV in the lineage C [5]. Beta coronaviruses in animals have a potential to infect human as in SARS and MERS and additionally have a potential to result a pandemic as in SARS-CoV-2.

Although CoVs are common causes of hospital and community acquired respiratory infections, they are understudied compared to influenza viruses [4]. Many microbiological laboratories do not identify CoVs. That's why the World Health Organization (WHO) has highlighted the importance of non-influenza respiratory viruses including CoVs [4,6].]. Like other respiratory viruses CoV infection peak incidence is noted in the winter season [7,8]. While OC43 is predominant in Canada and southeast Asia, HKU1 is frequent in the midwestern USA [4,9-11]. Newer members of CoVs; Severe acute respiratory syndrome SARS-CoV was first described in 2002 and Middle Eastern respiratory syndrome MERS CoV in 2012 [3]. The last member of human CoVs, SARS-CoV-2 was first identified in China in late December 2019. Since 2020, a pandemic caused by SARS-CoV-2 has been affecting the world. Common symptoms of SARS-CoV-2 infection are respiratory symptoms, fever, cough, and dyspnea. More severe cases were associated with lower respiratory tract infection, pneumonia, severe acute respiratory infection, kidney failure [3,12,13]. SARS-CoV-2 pandemic has highlighted the need to understand the epidemiology of CoV infections. In this retrospective study, our aim was to analyze the incidence rate of CoVs 229E, NL63,OC43 and HKU1 with use of Real-Time(RT) PCR in respiratory samples of patients hospitalized in our hospital between April 2018 and March 2020 to have a better understanding of CoV epidemiology.

2. MATERİALS AND METHODS

Bursa Uludag University Hospital is a reference centre at the South Marmara Region with a population of about five million. In this study, hospitalized patients with respiratory symptoms including nasal congestion, rhinorrhea, nasal discharge, sneezing, sore throat and cough, were tested for respiratory viruses including CoVs 229E, NL63,OC43 and HKU1 between April 2018 and March 2020 and the results of these patients were analyzed retrospectively.

Results of nasopharyngeal swab PCR between 04.01.2018 and 03.31.2020 were analyzed. FLOQ Swabs (Copan, Italy) were used for nasopharyngeal sampling and placed in a transport media (UTM-RT, Copan, Italy) and stored at -80°C. The samples were analyzed within four days.

American Thoracic Society (ATS) clinical practice guidelines were used to obtain BAL with fiberoptic bronchoscopy [14,15]. BAL specimens were obtained according to guidance of computed tomography. The samples were analyzed within four days.

We used Fast Track Diagnostics (FTD) respiratory pathogens 33 (Fast-track Diagnostics, Malta) kit for RT-PCR, in Rotor-Gene Q (Qiagen, Germany) between April 2018 and December 2019. We used QIAStat Dx Respiratory panel(Qiagen, Germany) in QIAStat Dx (Qiagen,

Germany) for detection of respiratory viruses between January 2020 and March 2020. QIAStat Dx (Qiagen, Germany) combines nucleic acid extraction and RT-PCR in the same device by a cartridge. Both of the kits included coronaviruses (CoVs) NL 63, 229E, 0C43 and HKU1.

We included the results of the samples including CoV positive respiratory samples. Respiratory samples with negative samples ot positive other than CoV was excluded. The results of FTD respiratory pathogens 33 (Fast-track Diagnostics, Malta) kit were interpreted according to the manufacturer's instructions; if the cycle threshold(Ct) was ≤35 in the presence of a sigmoid curve the result was positive. PCR results were analyzed according to MIQE guidelines key criteria chart [16]. QIAStat Dx Respiratory panel (Qiagen, Germany) tests were analyzed by the device software and results were reported according to the instructions of the manufacturer.

IBM SPSS Statistics v20 programme (SPSS INC, Chicago, USA) was used for statistical analysis. We used chi-square test on SPSS for statistical analysis. A p value of *P* =.05 or less was accepted as significant.

3. RESULTS AND DİSCUSSİON

We retrospectively analyzed the results of 1422 respiratory samples (790 – 55.6% from male patients and 632 – 44.4% from female patients) including 1245 nasopharyngeal swabs and 177 BAL samples. The patients were between 0-93 years with a mean age of 26 years. Most of the patients (957 (76,9%)) were ≤18 years. Mean age of patients with upper respiratory tract infection was 18,2 years. There were 693(55,7%) male patients with upper respiratory infection symptoms and 97 (54,8%) males in the lower respiratory infection group. BAL collected patients were aged between 18 and 88 with a mean age 53,5 years.

We detected 142 (11,4%) cases of CoVs in 1245 nasopharyngeal samples and 21 (11,9%) in 177 BAL samples. CoVs were positive in 110 (11,1%) of 993 of respiratory samples collected from individuals ≤18 years and 53 (12,4%) of 429 individuals >18 years (Table 1). There was no statistically significant difference between age groups for CoV positivity. CoV subtypes are listed in Table 2 both for nasopharyngeal and BAL samples. There was no statistically significant difference between the positivity of nasopharyngeal and BAL samples for CoVs. The

commonest CoV was OC43 (n=72, 44,2%) in overall samples. While 68 of 142 positive nasopharyngeal samples were only positive for CoVs, 74 were positive for other respiratory viruses besides CoVs. Accompanying viruses in nasopharyngeal samples were rhino/enterovirus (n=25), influenza (n=21) and respiratory syncytial virus (RSV) (n=16) . Although 9 of BAL samples were positive for only CoVs, 12 were positive for at least two viruses and common accompanying viruses were influenza (n=8) and rhinovirus (n=3) (Table 3).

CoVs were usually detected in the winter season between December and March (Fig. 1). Positivity rate of CoVs among samples according to months were highest in January 2019 as 34 positives with the positivity rate 38,6%. There were 16 positive samples in January 2020 which means a total of 50 CoV positivity in the January which is higher than the other months. January is the coldest month of the year in Turkey which covers the 30,7% of all CoV positivity in our hospital.

The CoV-229E, -OC43, -NL63 and -HKU1 are endemic all around the world. CoVs usually infect the upper respiratory tract in the healthy people without underlying diseases, and they are mainly associated with 15–30% of common cold infections [17]. Although CoVs are highly opportunistic, they can infect the lower respiratory tract leading to more life threatening infections like bronchitis, bronchiolitis, pneumoniae and respiratory distress distress syndrome [4,12,17,18].

Beta CoVs are emerging viruses since 2002. SARS resulted in a pandemic beginning in 2002 [19]. More than 8000 probable cases were reported and approximately 10% (774 cases in more than 30 countries) of these cases died because of the SARS CoV[17,20]. In the last quarter of 2012, travellers from the Arabian Peninsula were infected by the Middle-East Respiratory Syndrome (MERS) CoV. MERS CoV was another CoV leading to severe acute respiratory distress syndrome and additional symptoms of the gastrointestinal system and renal findings among some patients[21]. MERS-CoV which is a betacoronavirus, was found in an intermediary reservoir (the dromedary camel). Dromedary camel which transmitted the virus to humans, was possibly infected by bats. Additionally, nosocomial transmission was observed in many hospitals in the Arabic peninsula [21,22]. While no SARS infection was

detected in Turkey, only one MERS patient who returned from Saudi Arabia, was detected in Turkey [23]. Many cases representing with lower respiratory infections in Wuhan, China, was because of a novel betacoronavirus, SARS-CoV-2. The virus which has a zoonotic transmission to humans, infected millions of individuals around the world and Turkey has been also affected by the SARS CoV-2 [3,12,23]. Analyzing the epidemiological and virological characteristics of endemic CoVs may help to understand the dynamics of SARS CoV-2. Gaunt et al.[24] analyzed the burden of endemic CoVs using a multiplex Real-Time PCR. They analyzed 11661 respiratory samples and found CoV less than 1% in their tests [24]. This study was done in 2010 with an in-house assay which is supposed to the low detection rate of CoVs according to our study which means that technique of obtaining a respiratory sample and/or the PCR kit is responsible for significantly lower incidence rate of CoVs in study by Gaunt et al. [24]. The seasonality of CoVs in the United States of America (USA) is similar to our findings between December and April which is due to the fact that both the countries are in the northern hemisphere [7,24]. CoV OC43 was the commonest CoV detected in the USA in different studies similarly to our results [7,24].

Our findings about endemic CoVs suggest that, they are one of the commonest respiratory viruses circulating in the world. We found a positivity rate for CoVs in upper respiratory samples 11,4% and lower respiratory tract samples 11,9% similar to the rate 11,3% of all respiratory samples, found by Kozak et al.[4] Additionally, the commonest CoVs found by Kozak et al.[4] was CoV OC43, followed by 229E, the same as our result [4].

We found that most of the CoVs were a part of co-infections. Co-infection rate was 45,7% in nasopharyngeal samples and 57,1% in BAL samples. Most of the co-infections were accompanying with influenza and rhinoviruses.

Matsuno et al.[25] investigated children and found that respiratory viral co-infections were common in the children with a ratio of 78% for overall viruses. They also found that co-infections of CoVs were common with rhinoviruses. Matsuno et al.[25] pointed at the importance of CoV infection alone or co-infection with rhinovirus is a risk factor for severe respiratory disease. Also in Brasil the commonest CoVs were OC43 followed by 229E [25].

CoVs may be the cause of other systematic infections than the respiratory tract. Common CoVs are possible epidemiological agents for extra respiratory findings such as myocarditis, severe diarrhea, meningitis and multi-organ failure especially in in children [17,26]. Recent studies showed that all CoV strains can be found in gaita of patients with acute gastroenteritis; however, no evidence of association could yet be clearly demonstrated with disease etiology [27,28]. Different reports also presented a possible link between the presence of CoVs in central nervous system (CNS) and some neurological disorders among patients examined. Like all viruses, CoV may enter the CNS through the hematogenous or neuronal retrograde route [17,29].

Our study shows that alpha and beta CoV infections are predominant in the winter season but during all the year with lower impact in the summer. Wu et al.[13], analyzed the effect of temperature and humidity on the daily new cases and new deaths of COVID-19 and found that, 1°C increase in temperature was associated with a 3.08% reduction in daily new cases and a 1.19% reduction in daily new deaths, whereas a 1% increase in relative humidity was associated with a 0.85% reduction in daily new cases and a 0.51% reduction in daily new deaths. [30]. There is not yet data about the co-infections of SARS CoV-2 and co-infecting other virus may also be life-threatening besides SARS CoV-2. So, we argue that other respiratory viruses and SARS

Table 1. Number of test requests and CoV positivity according to age groups and sample type

Age (years)	NP req (n)	BAL req (n)	Total req (n)	NP CoV (n)	BAL CoV (n)	Total CoV (n)
0-1	109	0	109	11	0	11
2-5	520	0	520	62	0	62
6-18	364	0	364	37	0	37
19-65	184	132	316	26	19	45
>65	68	45	113	6	2	8
Total	1245	177	1422	142	21	163

CoV: Coronavirus, n: number, NP: nasopharyngeal, BAL: broncho alveolar lavage, req: Test request
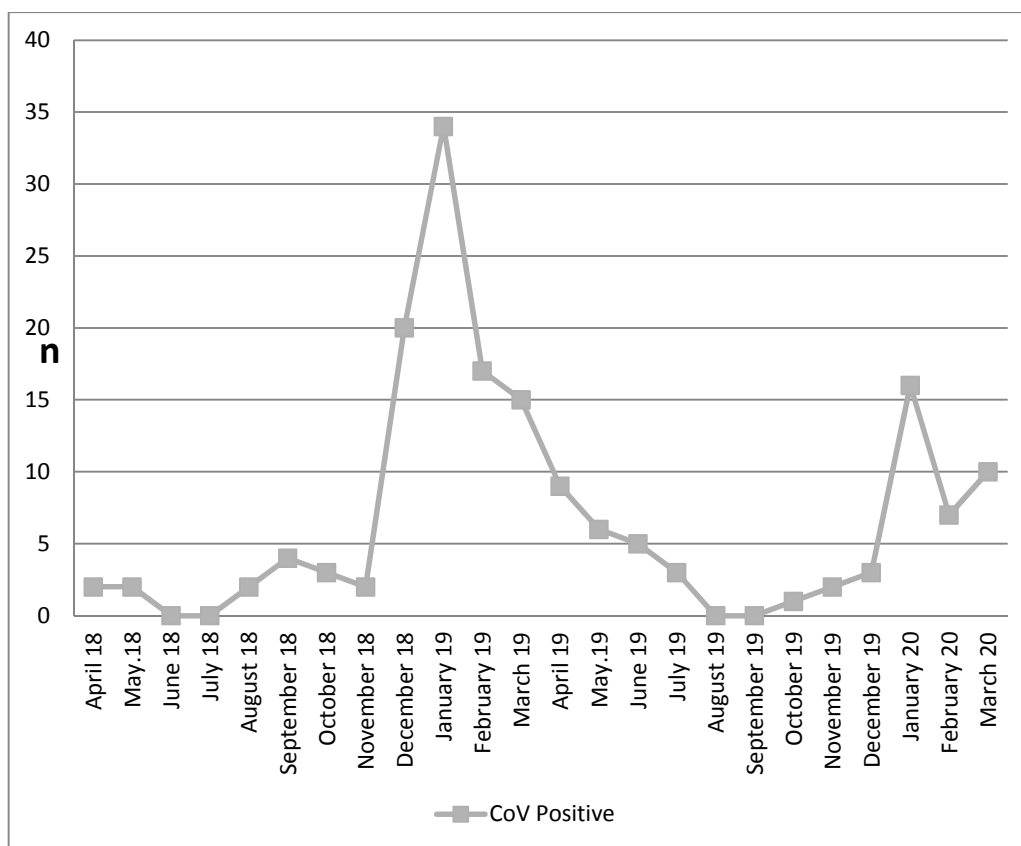
Fig. 1. Number of CoV positive samples according to months (n: Number)

Table 2. Coronavirus subtypes and numbers in nasopharyngeal and BAL samples

Subgroup	CoV Subtype	NP	BAL	Total
Alpha	229E	42	11	53
	NL63	18	2	20
Beta	OC43	67	5	72
	HKU1	15	3	18

CoV: Coronavirus, NP: nasopharyngeal, BAL: broncho alveolar lavage

Table 3. Other viruses in mixed viral infections accompanying CoVs

Virus	NP	BAL	Total
Influenza	21	8	29
Rhino/enterovirus	25	3	28
RSV	16	1	17

CoV: Coronavirus, NP: nasopharyngeal, BAL: broncho alveolar lavage, RSV: respiratory syncytial virus

CoV-2 should be analyzed by high quality commercial multiplex Real-Time PCR kits.

Our results show that beta CoVs are more frequent than alpha CoVs in our region (Table 2). Beta CoV OC43 was usually detected in nasopharyngeal samples. We detected 21 CoVs in BAL samples which means that 11,9% of CoV infections are affecting lower respiratory tract. Lower respiratory viral infections are an important cause of mortality and morbidity but new and rapid Real-Time PCR tests quickly show the causative agent which is important for specific treatment [31,32]. This study has a limitation; data was collected from single center.

4. CONCLUSİON

Both alpha and especially beta human CoV infections are very important due to the potential of mutations resulting in epidemics. Since 2002 beta CoVs are leading to epidemics and the latest one SARS CoV-2 resulted in a pandemic.

Although CoV infections are predominant in the winter season, they are diagnosed throughout the year with lower incidence in summer and are identified in individuals of all ages. Understanding the epidemiological and virological features of CoV infections is important to effectively control their burden.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHİCAL APPROVAL

All authors hereby declare that the study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/65236