Investigation of respiratory tract coinfections in Coronavirus disease 2019 infected and suspected cases

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ABSTRACT

OBJECTIVE: The aim of our study is to determine the risk of coinfection with COVID-19 due to the high prevalence of viral agents in Istanbul in autumn (September, October, and November) and winter (December and January) and to investigate the effects of age, gender, season and clinical features on the development of coinfection with COVID-19.

METHODS: In the routine studies of our hospital, COVID-19, reverse transcriptase polymerase chain reaction (RTA kit, Turkiye) and Multiplex PCR Bio-Fire (Bio Merieux Company, France) methods were studied from the nasopharyngeal swab sample and the data were recorded. A total of 400 people with a mean age (7.91±17.80) were included in the study by retrospective scanning.

RESULTS: Considering the virus distribution, Respiratory syncytial virus (RSV), COVID-19, rhino/entero virus did not show a significant difference in autumn and winter, while H. metapneumovirus, adeno virus, influenza A significantly higher rates were observed in winter months. Parainfluenza (1, 2, 3, 4) and Corona OC43 were detected at a higher rate in autumn compared to other viruses. Double and triple coinfection rates with other viral agents were high for 2 years and younger.

CONCLUSION: The risk of coinfection of COVID-19 with influenza A, RSV, parainfluenza, and rhino/entero virus was found to be higher than other viral agents. Especially in winter, the risk of coinfection with influenza A and COVID-19 increases. In terms of treatment management, coinfection should be investigated in risky patients and influenza a vaccine should be offered to risky groups.

Keywords: Coinfection; COVID-19; multiplex PCR.

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kits containing other viruses, they could not be used for screening purposes. Approximately 5000 tests/day COVID-19 reverse transcriptase polymerase chain reaction (RT PCR) were studied in our hospital, which served as the center of the pandemic in Türkiye, Istanbul. Considering that it will be seen frequently in other viruses in autumn and winter months, a viral respiratory panel has been added to the routine. The data of the patients studied in the routine were evaluated. Common cold coronaviruses that cause respiratory tract infections are HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, four endemic HCoVs, they use different receptor molecules with varying host cell tropism [1]. SARS-CoV-1 and MERS-CoV SARS-CoV-2 are zoonotic emerging epidemic pathogens that cause significant morbidity and mortality. Endemic HCoVs are known to cause coinfections or can be detected with each other or with other respiratory viruses. The distribution of respiratory virus agents differs between countries, states and even cities. Coinfections with influenza A, influenza B, adenovirus, parainfluenza virus, Human metapneumovirus, bocavirus, respiratory syncytial virus (RSV), rhino/enterovirus, Ebstein-Barr virus (EBV), and Cytomegalovirus (CMV) have been reported in studies. Knowing the coinfection is of high importance in the diagnosis, follow-up and treatment of the disease [2–5].

The aim of our study was to investigate the presence of coinfection in patients whose multiplex PCR respiratory panel studied in the autumn and winter of 2021–2022, and to investigate virus infections and coinfections that are common in our region and to evaluate the effects of age, gender, clinic and season on the occurrence of coinfection.

MATERIALS AND METHODS

Study Design
Viral agents and demographic data determined by the syndromic panel in autumn and winter were reviewed retrospectively. Age, gender, and initial complaints of the patients were recorded.

Detection of COVID-19 with RT PCR
The COVID-19 detection was performed by the COVID-19 qPCR test (Direct Detect RTA, Türkiye) on BioRad CFX 96 platform (California, USA) according to the protocols provided by the manufacturer. The qPCR kit targets ORF1ab and the N gene of SARS-CoV-2 and the human RNaseP gene.

Detection of Respiratory Pathogens with Syndromic Panel
By using BioFire FilmArray Multiplex PCR System (BioMerieux, France) Adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, middle east respiratory syndrome coronavirus (MERS-CoV), Human metapneumovirus, Human rhinovirus/enterovirus, influenza A, influenza B, parainfluenza virus 1, parainfluenza virus 2, and parainfluenza virus 3 viruses were studied. In addition to, parainfluenza virus 4, RSV virus, Bordetella parapertussis, Bordatella pertussis, Chlamydia pneumoniae, Mycoplasma pneumoniae among the bacteria found on the panel of the device were investigated.

Statistical Analysis
NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the study data, descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, and maximum) were used for groups with normal distribution, independent sample t-test, and for comparison of groups not showing normal distribution, Kruskal-Wallis test was used. Logistic regression analysis was used in the impact analysis. Significance was assessed at levels of p<0.05.

RESULTS
The study was conducted with a total of 400 subjects, 57.5% (n=230) male and 42.5% (n=170) female, with a mean age of 7.91±17.80. When their age is examined; 2 years and younger; 66.5% (n=266), 3–10 years old; 15.5% (n=62), 11–18 years old; 6.8% (n=27), 19 years and over; 11.3% (n=45). The average age is 7.91 years. Min–Max (median): 0–91(1). Detected virus rates: RSV; 119 cases, rhino/entero; 155 cases, COVID-19; 96 cases, parainfluenza (1,2,3,4); 30 cases, Corona 29E;4 cases, Corona HKU1; 1 cases, Corona NL63;1

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Highlight key points
- Coinfection with COVID 19 and other viruses is particularly common in pediatric patients.
- Respiratory panel should be used in the evaluation of risky patient groups.
- Considering that Influenza A and COVID 19 coinfection may also be severe, Influenza A vaccination should be recommended to risky groups.
cases, Corona OC43; 12 cases, Adeno virus; 21 cases, H. metapneumovirus; 21 cases, influenza A; 22 cases. The number of negative detected cases is 40. When coinflection is evaluated; 32.5% (n=80) in dual coinfec-

Figure 1. Triple coinfection peak of COVID-19.

tion; triple coinfection 6.8% (n=18), dual coinfection with COVID-19:5.7% (n=15) triple coinfection with COVID-19:4.5% (n=12). Peaks of a patient with triple coinfection are visible in Figures 1–3. Figure 1;
peak of COVID-19, Figure 2; peak of RSV, and Figure 3; peak of rhino/entero virus. The distribution of cases is given in Tables 1 and 2. Gender has no effect on the distribution of viral infections (p>0.05), RSV, COVID-19, Rhino/entero virus did not show a significant difference in autumn and winter, H. metapneumovirus, adeno, influenza A was significantly higher in winter, parainfluenza and Corona OC43 were significantly higher in autumn (p<0.05). When age is evaluated, RSV is significantly more common under 2 years
of age, rhino/entero virus is significant at 3 years old and above, Corona OC43 is significant in 11–18 age group, COVID-19 is significant in 19 years old and above (p<0.05). When evaluated in terms of clinical upper and lower respiratory tract infections, while there was no significant difference in other viruses, admission with COVID-19 upper respiratory tract infection symptoms was found to be significantly higher (p<0.05) (Table 3) evaluation of the effects of other viral agents on season, gender, age, and clinical COVID-19 Logistic regression analysis (Table 4).

**DISCUSSION**

There are limited data on coinfection since the beginning of the pandemic. In our study, in addition to dual coinfections, triple coinfections were detected especially in newborns and children (Table 3). In COVID-19 infection, lymphocytopenia, overexpression of inflammatory cytokines, and dysfunction of the acquired immune system may predispose to the development of coinfection, which may result in harmful sequelae in a healthy individual [3, 6]. Given the fact that more than one mechanism is involved in the emergence of coinfections, it can be assumed that the mere presence of one virus and its effect on the immune system may serve as the basis for the replication and suppression of the other virus [6, 7].

Coinfection can be detected at the first application of the disease as well as later. A nasopharyngeal swab was taken for a respiratory pathogen panel as well as COVID-19 RT-PCR in a patient who presented to the emergency department with cough and shortness of breath. In a study, the respiratory pathogen panel was found to be Human metapneumovirus positive and the patient was treated and discharged. COVID-19 RT PCR was positive after 24 hours. This case reflects that COVID-19 testing algorithms that exclude patients who routinely test positive for viral pathogens may miss patients co-infected with COVID-19 [8].

The distribution of endemic viruses differs from region to region. From June 2020 to January 2021 in India, other respiratory pathogens were detected in 33 of 191 patients with COVID-19. The coinfection rate of these, with human adenovirus and human rhinovirus being the most common, was found to be 7.3% [3].

### Table 2. Distribution of coinfections

| Binary coinfections                  | n  | Triple coinfections          | n  |
|-------------------------------------|----|------------------------------|----|
| RSV+Rhino/entero                    | 35 | Paraifluenza+Corona OC43+Rhino/entero | 2  |
| RSV+Parainfluenza                   | 2  | COVID-19+RSV+Rhino/entero    | 5  |
| RSV+COVID-19                        | 3  | H. Metapneumo+COVID-19+RSV   | 1  |
| RSV+Adeno                           | 2  | Paraifluenza+Adeno+Rhino/entero | 1  |
| RSV+Influenza A                     | 1  | Paraifluenza+Influenza A+Rhino/entero | 1  |
| RSV+H. metapneumo                   | 1  | Paraifluenza+SARS CoV2+Rhino/entero | 2  |
| H. metapneumo+Influenza A           | 1  | COVID-19+Adeno+Rhino/entero  | 3  |
| H. metapneumo+Rhino/entero          | 3  | RSV+Parainfluenza+Corona 229E | 1  |
| H. metapneumo+Corona 229E           | 1  | RSV+Adeno+Influenza A        | 1  |
| COVID-19+Influenza A                | 1  | RSV+Adeno+Rhino/entero       | 1  |
| COVID-19+Rhino/entero               | 10 | RSV+Parainfluenza+Rhino/entero | 1  |
| COVID-19+Corona OC43                | 1  | H. metapneumo+COVID-19+Adeno | 1  |
| Adeno+Influenza A                   | 1  | H. metapneumo+Adeno+Rhino/entero | 1  |
| Adeno+Rhino/entero                  | 4  | H. metapneumo+COVID-19+Rhino/entero | 2  |
| Influenza A+Rhino/entero            | 3  |                             |    |
| Parainfluenza+Rhino/entero          | 10 |                             |    |
| Rhino/entero+Corona HKU1            | 1  |                             |    |
| Total                               | 80 |                             | 18 |

RSV: Respiratory syncytial virus.
In a study conducted in China, Mycoplasma and RSV were the most common coinfections in patients with COVID-19 pneumonia and were seen among rhino/entero, RSV, and other coronavirus species. Increased procalcitonin levels in patients with COVID-19 pneumonia have been associated with coinfection [9].

Coinfections in patients infected with COVID-19 in the greater metropolitan area of New York City have been identified as common respiratory viruses co-occurring with rhinovirus/enterovirus, Influenza viruses and coronavirus NL63 and other coronavirus family in the community. Additional studies are needed to determine whether concomitant viral infection could potentially lead to viral interference or affect disease outcomes in COVID-19 patients [10].

In our study, rhino/entero, RSV, parainfluenza, coronavirus OC43 emerged predominantly in the autumn of 2021 in Istanbul. Influenza A was seen in one case. It was observed that rhinovirus, RSV, coronavirus OC43, and parainfluenza coinfections seen with COVID-19 did not increase mortality and morbidity when compared to other patients. In other publications, there are data showing that influenza B, which increases mortality mostly by influenza A, increases chronicity [11, 12]. In our study, it was determined that the risk of co-occurrence of influenza A and COVID-19 is higher than other viruses. However, a clinical interpretation could not be made. Influenza B was not seen. It has been determined that influenza coinfection can trigger pus with COVID-19 hyperinflammatory state and cardiac effects are more. However, it may be difficult to detect COVID-19 mortality differences as the available data are only for critically ill patients [13, 14]. Studies have found that coinfections are more common than expected [10]. Currently, we
have little understanding of the viral kinetic parameters of COVID-19 infection, which makes it difficult to determine the dynamics of coinfection. It has been shown that viruses trigger competitive advantage between different mechanisms by which immune responses are elicited [10].

Studies have also shown that clinical symptoms and transmission dynamics are quite similar in patients with only COVID-19 infection and patients with influenza virus coinfection [15, 16]. It has been found that bacterial coinfections are more mortal than viral coinfections [17].

EBV should also be investigated in high-risk groups such as immunocompromised and transplanted patients. EBV coinfection causes an increase in mortality [18]. Severe lymphocytopenia occurs in severe COVID-19 infection, leading to cellular immune system deficiencies and consequent CMV re-infection or reactivation. Subsequently, disruption of the reticuloendothelial and hematopoietic systems such as the bone marrow, spleen, and lymph nodes may result in the death of T cells in severe COVID-19. In this context, as the population of effector and CD8+ cytotoxic T cells decreases, the effects of COVID-19 infection become more serious, leading to innate and adaptive immune system failure, and ultimately predisposing individuals to viral infections [19–21].

COVID-19 and adenovirus coinfection may pre-dispose the patient to mechanical ventilation by initiating a series of deleterious sequelae such as lymphopenia, thrombocytopenia, and septic shock. To date, coinfection of adenovirus and COVID-19 has been reported in limited numbers [22–24]. In addition, coinfections increase the spread of viruses. While the period of spread of the virus in influenza-related diseases is 5–10 days, it is 2–5 weeks in COVID-19 infection [25, 26].

A better understanding of the prevalence of coinfection with other respiratory pathogens and the profile of the pathogens in COVID-19 patients may contribute to effective patient management and management of therapy during the current pandemic [27].

The inadequacy of traditional methods in detecting coinfection and the lack of sufficient evidence may lead to underdiagnosis [3]. In patients with underlying disease, coinfection should be investigated [28].

| Step 1 |
|--------|
|        |
|        |
| B      | SE   | Wald  | Sig.  | ODDS | 95% CI for EXP(B) |
|--------|------|-------|-------|------|------------------|
|        |      |       |       |      | Lower | Upper |
| RSV(1) | 2.576 | 0.447 | 33.280 | 0.000 | 13.146 | 5.479 | 31.544 |
| H. metapneumovirus(1) | 0.966 | 0.556 | 3.018 | 0.082 | 2.627 | 0.884 | 7.808 |
| Parainfluenza (1) | 1.644 | 0.693 | 5.618 | 0.018 | 5.174 | 1.329 | 20.143 |
| Adeno virus(1) | 0.601 | 0.654 | 0.844 | 0.358 | 1.824 | 0.506 | 6.577 |
| Influenza A(1) | 3.605 | 1.095 | 10.834 | 0.001 | 36.794 | 4.299 | 314.881 |
| Rhinovirus(1) | 1.738 | 0.359 | 23.473 | 0.000 | 5.684 | 2.814 | 11.481 |
| Seasons (1) | -0.445 | 0.361 | 1.519 | 0.218 | 0.641 | 0.315 | 1.301 |
| (0–2) age | 6.791 | 0.079 |
| (3–10) age | -1.051 | 0.515 | 4.165 | 0.041 | 0.350 | 0.127 | 0.959 |
| (11–18) age | -1.364 | 0.589 | 5.366 | 0.021 | 0.256 | 0.081 | 0.811 |
| (19<) age | -1.354 | 0.658 | 4.233 | 0.040 | 0.258 | 0.071 | 0.938 |
| Gender(1) | -0.127 | 0.296 | 0.184 | 0.668 | 0.881 | 0.493 | 1.574 |
| Clinic(1) | 0.648 | 0.346 | 3.507 | 0.061 | 1.911 | 0.970 | 3.765 |
| Constant | -9.728 | 1.851 | 27.635 | 0.000 | 0.000 | 0.000 |

Variable(s) entered on step 1: Gender, RSV, H. metapneumovirus, parainfluenza, adeno virus, influenzaa, rhino/entero, season, age, clinic. SE: Standart error; ODDS: Risk ratio (OR); CI: Confidence interval; EXP: Exponansiyel beta.
Conclusion
As can be seen, coinfections are substantial and their presence increases the duration of transmission. Mechanisms of action differ according to the difference of viruses, and mortality and morbidity change. It is also necessary to develop new strategies to better understand the clinical manifestations of coinfections and explore appropriate treatment options for them. Accordingly, early detection of coinfections is important because of differences in treatment and favorable prognosis. It should be routinely investigated in newborns and children, especially in the case of immunodeficiency, and the treatment should be planned accordingly. In our study, it was determined that the risk of COVID-19 coinfection with the increase of influenza A virus in winter may be higher than other viruses. Therefore, the risk group should be included in the influenza a vaccine program, which is revised every year. Further studies are needed in terms of underlying mechanisms and treatment protocols.

Ethics Committee Approval: The Health Sciences University Kanuni Sultan Suleyman Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 14.10.2021, number: 2021.10.252).

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