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Original Article

High frequency of co-infection between SARS-CoV-2 and respiratory Adenoviruses in the Pediatric population in Hamadan, Iran

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Abstract

Introduction: The presence of other respiratory pathogens in patients with SARS-CoV-2 infection has been described as a striking feature. However, data on adenovirus co-infection rates and clinical impacts in COVID-19 patients is limited. The purpose of this study is to compare the prevalence of respiratory adenoviruses in children under 15 years of age in Positive and Negative SARS-CoV-2 patients.

Methodology: From September 2020 to January 2021, nasopharyngeal swabs were obtained from 280 patients below 15 years old with influenza-like infection symptoms suspected to be COVID-19 and referred to hospitals in Hamadan province. Nucleic acid was extracted using a High Pure Viral Nucleic acid extraction kit for both viral RNA and DNA. Reverse transcription real-time PCR for detecting SARS-CoV-2 and Real-time PCR for Human Adenoviruses were used.

Results: Out of 280 examined samples, 11.7% tested positive for AdV, of which 18 samples originated from the SARS-CoV-2 positive group and 15 samples were from the SARS-CoV-2 negative group. Of 18 co-infected samples, which were categorized in three different ranges of age including, 0-5, 6-10, and 11-15 years old were 11, 4, and 3 patients respectively. Also, 14 patients were hospitalized. Compared with AdV-positive patients, children with Co-infection with SARS CoV-2 had lower levels of white blood cell (WBC) count while erythrocyte sedimentation rate (ESR) and C-reaction protein (CRP) had increased levels.

Conclusion: We report a substantial burden of AdV co-infection in pediatric COVID-19 patients. This study revealed most AdV infections lead to hospitalization and change in *paraclinical* parameters.

Keywords: SARS-CoV-2, Adenoviruses, Co-infection

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Introduction

Coronavirus illness 2019 (COVID-19) is an illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of December 11, 2022, there were more than 653 million confirmed cases and 6,658,478 deaths around the world, counting more than 7,560,000 cases and 144,658 deaths in Iran. Since the onset of the pandemic, fewer cases of symptomatic COVID-19 have been reported in children worldwide and very few cases have required hospitalization, however, children cases spiked dramatically in 2022 during the highly transmissible Omicron variant emergence. It is well established that contamination with one respiratory pathogen makes a patient vulnerable to secondary infections through a few instruments, counting epithelial damage. Respiratory viruses are the most common cause of respiratory tract infections (RTI) among children. The most common viral agents are respiratory syncytial virus (RSV), influenza viruses, human rhinovirus (hRV), enteroviruses (EV), coronavirus (CoV), adenovirus (AV), parainfluenza viruses (PIV 1-4), human metapneumovirus (hMPV) and human bocavirus (hBoV). Human adenoviruses (HAdVs) play a noteworthy part in pediatric acute respiratory contamination, accounting for 5–10% of all respiratory tract infections in children including pharyngitis, tonsillitis, pharyngo-conjunctival fever, bronchitis, and pneumonia. In expansion, HAdV inactive contamination in lymphoid tissue was depicted and reactivation with asymptomatic carriage may occur. Recent studies have shown that mixed respiratory viral infection might result in improved or decreased disease severity compared with single infections. At the host level, synergistic or antagonistic virus–virus interactions might result in an increased or decreased pathogenesis and good or poor disease outcomes. There's a shortage of information concerning the comparison of laboratory and clinical characteristics between HAdV and SARS-CoV-2 infection among pediatric patients.

Adenoviruses may be considered an important agent in co-infection with COVID-19. In a study, a person infected by SARS-CoV-2 and respiratory adenovirus concurrently had increased dyspnea, tachypnea, and desaturation despite supplemental oxygen, so he was transferred to the intensive care unit. The prevalence rate of adenoviruses differs for example in a study in Senegal of 6381 samples tested, 1967 (30.8%) tested positive for HAdV, and 1561(79.4%) cases were co-infected with other respiratory viruses. Adenoviruses are non-enveloped, icosahedral viruses with linear double-stranded DNA genomes.

Evaluation of the epidemiology and aetiology of seasonal respiratory viruses in patients with COVID-19 may determine the rate of SARS-CoV-2 co-infection to better understand the role of such viruses in patients' clinical symptoms. This could improve the patient treatment process and further contribute to public health practices. The objectives of this study were to rate co-infection between SARS-CoV-2 and respiratory Adenoviruses in children up to 15 years of age in Hamadan, Iran to describe and compare the possible correlations between age, gender, and hospitalization status of individuals with co-infection.

Methodology

Test specimens.

At the 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 15 years) with respiratory symptoms from September 2020 to January 2021. Nasopharyngeal samples were collected in 3 - 4 ml of transport medium and kept at -70°C until testing. The abundance of patients in three age groups 0-5, 6-10, and 11-15 years old were 207, 37, and 36 children respectively. The number of Males and females was 163 and 117 respectively.

Table 1: Distribution of patients

	Age range (years)			Genus		COVID-19	
	0 – 5	6 – 10	11 – 15	Male	Female	Positive	Negative
No. of patients	207	37	36	163	117	140	140

Genomes Extraction

Nucleic acid was extracted from 200 µl of the sample using a highly pure Viral Nucleic acid extraction kit (Roche, Germany) for both viral RNA (SARS-CoV-2) and DNA (Adenoviruses) according to the manufacturer's instructions briefly, Binding buffer, Proteinase K and Sample were combined and incubated. Then we inserted the High Pure Filter Tube into the Collection Tube and after centrifugation, we added the Inhibitor and washed it twice with Wash Buffer. In the last stage added Elution Buffer. Centrifugation was performed in each step.

Reverse transcription real-time PCR for detecting 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(13).

The table below shows the Ct cut-off for each fluorescent channel.

Target	Ct Value
ORF1ab gene	Ct ≤36
E gene	Ct ≤36
qaIC	Ct ≤32

Real-time PCR for Human Adenoviruses

The AdV primers and probe sequences were as follows: forward primer, 5'-GCC CCA GTG GTC TTA CAT GCA CAT C-3'; reverse primer, 5'-GCC ACG GTG GGG TTT CTA AAC TT-3'; and probe, 5'-FAM-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-BHQ1-3' (14). individual RT-qPCR assay was performed on an Applied Biosystems® 7500 Real-Time PCR system (Life Technologies™), using the AgPath-ID™ One-Step RT-PCR (Life Technologies™) kit previously described by Kodani et all(15) but modified to use standard optimal thermocycling conditions for viral assays: 94°C for 60 s followed by 45 cycles at 94°C for 30 s and 60°C for one min.

Results

We studied 280 specimens tested for SARS-CoV-2 and Human respiratory Adenoviruses; 140 of the 280 specimens (50%) were positive for SARS-CoV-2 and 33 specimens (11.7%) were positive for Human respiratory Adenoviruses from the total of 280 samples. The assumption of normality of the distribution of the variables was checked using the Kolmogorov-Smirnov test and the normal distribution was established for all the variables. We used the student T-test for the comparison of a continuous variable between groups. Data were analyzed using SPSS-Version24, and the significant level was considered less than 0.05. Table 2 reports patient demographics and is stratified by the presence of SARS-CoV-2 and Adenoviruses pathogens.

Table 2: Demographics and classification of patient

Characteristic		SARS-CoV-2 status	
		Positive	Negative
No. of patients		140	140
Female No / total (%)		55(39.3%)	62(44.3%)
Hospitalization		78(55.7%)	71(50.7%)
age (range)	0 – 5	92(65.7%)	115(82.1%)
	6 – 10	29(20.7%)	8(5.7%)
	11 – 15	19(13.6%)	17(12.2%)
AdV No.		18	15

Table 3: Characterization of AdV Positive cases

		Total Number	AdV Positive	Percentage AdV/Total
No. of patients		280	33	11.7%
Female No/total		117	16	13.6%
age (range)	0 – 5	207	24	11.6%
	6 – 10	37	4	10.8%
	11 – 15	36	5	13.8%

Out of 33 patients with Adenovirus, there were 24, 4, and 5 people in the three age groups of 0-5, 6-10, and 11-15, respectively, which were 11.59%, 10.8%, and 13.8% of the total number in the three age

groups(P=0.91). Adenovirus patients have a hospitalization rate of 81%. Of these 33 people, 16 were girls and 17 were boys (Table 3). Out of 280 patients, 53% were hospitalized, while hospitalization in adenovirus-positive patients was 81%(P<0.001). Of the 140 specimens positive for SARS-CoV-2, 18 cases (12.8%) were positive for Adenoviruses, compared with 15 (10.7%) of the 140 specimens negative for SARS-CoV-2 (12.8% VS 10.7% P=0.58) (Table 1). Out of 140 positive SARS-CoV-2 samples, 39.3% were girls and out of 140 Negative SARS-CoV-2 samples, 44.3% were girls. Therefore, the occurrence of influenza-like symptoms was observed less in girls than in boys. The ratio of hospitalization in children with flu-like symptoms has a slight difference between positive and negative COVID-19 diseases. Table 2 shows the percentage of patients in three age categories, but the proportion of positive COVID-19 cases in age groups 0-5, 6-10, and 11-15 is reported as 44%, 78.3%, and 52.7%, respectively. This result shows that the prevalence of COVID-19 is higher in school age than in pre-school age.

Of 18 co-infected samples, which were categorized in three different ranges of age including, 0-5, 6-10, and 11-15 years old were 11, 4, and 3 patients respectively (P=0.66). Also, 14 patients were hospitalized. The gender distribution of 18 patients in the male and female group were 8 and 10 persons respectively (9.4% VS 18% P=0.12). Table 4 revealed the characterization of these 18 patients:

Table 4: Characterization of co-infection cases

	Age range				Genus	
	0 – 5	6 – 10	11 - 15	hospitalization	Female	Male
No. of patients	11	4	3	14	10	8
Proportion to participators	12%	13.7%	15.7%	77.7%	18%	9.4%

Comparisons of laboratory test results between co-infected and mono infected AdV patients

Laboratory tests were done on the day of hospital admission for the hospitalization. Compared with AdV-positive patients, children with Co-infection with SARS CoV-2 had lower levels of white blood cell (WBC) count while erythrocyte sedimentation rate (ESR) and C-reaction protein (CRP) had increased levels (table 5).

Table 5: Comparisons of laboratory test results between co-infected and mono infected AdV patients.

	AdV infected patients N=33 (mean)	AdV infection only N=15 (mean)	Co-infection group N=18 (mean)
WBC (10^9 /L)	9560	11020	8438
NEU (%)	60	60	60
LYM (%)	35	35	35
MON (%)	3	3.6	2.6
ESR	25.9	23.8	28
CRP	2	1.5	19

Figure 1: Characterization of co-infection cases

Figure legend text: Of 18 co-infected samples, which were categorized in three different ranges of age including, 0-5, 6-10, and 11-15 years old were 11, 4, and 3 patients respectively (P=0.66)

	Left figures 0-5 Years Old	Centric figures 6-10 Years Old	Right figures. 11-15 Years Old
SARS-CoV-2 Positive	92	29	19
Co-infected rate	12%	13.7%	15.7%

Discussion

The rate of viral co-infection has been previously reported to range from 2.0% to 19.8% in different countries (16-19). The most common viral co-infections reported are Enterovirus /rhinovirus up to 6.9 % and syncytial respiratory virus up to 5.2 % (16, 20).

Although there are few reports of SARS-CoV-2 with other respiratory viruses (20, 21), we found a significant rate of co-infection with Adenoviruses. This is in line with previous findings in the literature focusing on pediatric co-infections with COVID-19, the prevalence of co-infection was observed at 40-50% in children (22, 23). They published the prevalence of co-infection with SARS-CoV-2 between respiratory pathogens, and viral and atypical bacterial co-infections as 8.8%, 7.9%, and 0.9%, respectively (24). In France, a total of 4222 patients were tested for SARS-CoV-2 and other respiratory.

viruses and 3.7% of co-infected cases were Adenoviruses and their mean age was 59.6 ± 23.8 years (25). In China, between January 19, 2020, and February 26, 2020, Of the 250 hospitalized COVID-19 patients, 39 (15.6%) tested positive for at least one respiratory pathogen and co-infection of SARS-CoV-2 and Adenoviruses were 2.8% (26). The patients co-infected comprised 10 females (55%) and their mean age was 45 years. As might have been expected, our findings were a little contradictory to other studies mainly because we examined the prevalence of adenovirus in COVID–19–positive children younger than 15 years. Various studies have assessed co-infections in adults with COVID–19, but data on pediatric COVID-19 co-infection are limited. In our study, the rate of co-infection in three age ranges, 0-5, 6-10, and 11-15 were 12%, 13.7%, and 15.7% respectively. It can be due to increased communication with peers and fewer health protocols in this age category. However, this was not statistically significant between the two age ranges 0-5 and 6-15 with p =0.66. Gender has no impact on rates of co-infection (P= 0.12). Co-infection rates between SARS-CoV-2 and Human respiratory Adenoviruses increased with age.

Undetected co-infections may have severe clinical implications associated with increased hospitalization, varied treatment approaches, and mortality. Clinical manifestations of SARS-CoV-2 co-infection are not clear (21, 27). It has been documented in descriptive research that a higher proportion of the patients required hospitalization (20). More research consisting of nested PCR for respiratory germs is required to detect probably treatable pathogens (27). 18 cases of positive SARS and 15 cases of negative SARS have AdV, using t-Tests it was found that SARS-Cov-2 did not affect the AdV infection rate (12.8% VS 10.7% p=0.4).

78 cases of SARS-CoV-2 positive and 71 cases of SARS-CoV-2 negative needed hospitalization that was not statistically significant hospitalization (P=0.4). The limitation of our study is the inability to serotyping of Adenoviruses, which, if carried out, could detect the prevalence of a specific serotype.

However, the studies on simultaneous infections in the recent pandemic are limited. Among these studies, the age of children and the prevalence of Adenoviruses have been unintentionally neglected. Probably because our study was carried out in a cold region and in a cold season, which coincides with one of the COVID-19 waves in Iran, we could report a high incidence of respiratory infections in children, so a high degree of co-infection has been observed compared to other researches.

Limitations of the study

The limitations of our study include the impossibility of comparing the prevalence in different ages and comparing the prevalence in different seasons.

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Transparency declarations
There is nothing to declare.

Statement of informed consent: A written informed consent was obtained from participants before Sampling in accordance with ethical principles.

Statement of ethical approval: Ethical approval was given by the Hamadam University of Medical Sciences Ethics Committee (IR.UMSHA.REC.1400.213)

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