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Detection and Epidemiological Features of Human Coronaviruses in Patients with Acute Gastroenteritis in Northern Jordan

Mamdoh M. Meqdam[1,*], Malak I. Abusamha[2], Asem M. Alkhateeb[2] , Ziad M. Bataineh [3], Khadija F. Maho[1], and Ayat M. Gogazeh[1]

[1]*Applied Medical Sciences Faculty, Jordan University of Science and Technology;* [2]*Arts and Sciences Faculty, Jordan University of Science and Technology;* [3]*Medicine Faculty, Jordan University of Science and Technology*

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Abstract

Fecal specimens collected from patients with acute gastroenteritis among the Northern Jordan population were screened for human coronaviruses-229E, human coronaviruses-NL63, human coronaviruses-HKU1, and human coronaviruses-OC43 by Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) and PCR. Out of the 401 analyzed specimens, 42(10.5%) specimens were found positive for at least one human coronavirus. Of the 42 specimens, 57.1% were positive for human coronaviruses-229E, 33.3% for human coronaviruses-NL63, and 9.5% for human coronaviruses-HKU1. The human coronaviruses-OC43 virus was not detected in the tested specimens. None of the fecal specimens collected from healthy individuals were found positive for human coronavirus strains. No significant association was found between human coronavirus infection and gender (P>0.05). Most infected cases were in the age group >60 years old (23.8%), followed by the age group 0–1-year-old (19.0%). Most cases of human coronaviruses were detected in the winter season (42.9%) with a significant association recorded with human coronaviruses-NL63 (P = 0.006), and the lowest in the spring season (4.8%).The relationship between the human coronavirus-229E and fever (P = 0.04) and between human coronavirus-HKU1 and weakness (P = 0.04) were significant. No association (P> 0.05) between respiratory disease and positive human coronaviruses fecal specimens. The average symptom duration was 2-3 days. Among the viral-positive specimens, 38.1% were under antibiotic treatment. The provided data will help in patient care control of viral acute gastroenteritis.

Keywords: Gastroenteritis; Human Coronaviruses; RT-PCR; PCR; Jordan

1. Introduction

Acute gastroenteritis (AGE) is a diarrheal illness that could be associated with many clinical manifestations and death worldwide and the most AGEcases in developing countries were in young children (Kotloff , 2017, Bányai *et al.,* 2018, Lo Vecchio, 2020). Viruses are the leading cause of acute gastroenteritis globally, accounting for the majority of diagnosed acute episodes of community-acquired diarrhea. About 50-70% of AGE was caused by viral infections(Luo*et al.,* 2019). Today, it is regarded as a major cause of mortality and morbidity in children under 5 years old (De Francesco*et al*., 2021, Thwiny*et al*., 2022). Moreover, in 2013 diarrheal disease accounted for 9% of total deaths around the world (Liu*et al*., 2015). The World Health Organization defines diarrhea as three or more occurrences of loose or watery stools per day, for three days or more, and less than 14 days (WHO 2005).Enteric viruses causing AGE varied from watery diarrheal along with symptoms such asanorexia,vomiting, nausea, fever, or malaise, to severe dehydration that needs hospitalization or leads to death (Lee*et al.,* 2020, Xiong *et al.,* 2020). Enteric viruses causing gastroenteritis were rotaviruses (Gupta*et al*., 2019,Omatola and Olaniran*et al*., 2022), enteric adenoviruses (Biscaro *et al*., 2018), astroviruses (Vu*et al*., 2017), caliciviruses (norovirus and sapovirus)(Desselberger *et al*., 2017). New viruses, human bocavirus (Guido *et al.,* 2016), human coronavirus (Owusu*et al*., 2021), aichivirus (Chuchaona *et al*., 2017), klassevirus (Pei*et al.*,2016), salivirus (Lasure *et al*., 2016) were increasingly detected in AGE cases from different countries. As most enteric viruses cannot be grown in cell culture, electron microscopy is considered the mainstay of diagnosis(Zhao*et al*., 2019,Donelli *et al*., 2021). Applying more accurate methods for viral antigen detection in fecal specimens using immunoassay and molecular biology techniques has enhanced the diagnosis of new viruses causing gastroenteritis(Malik *et al*., 2019).

Human coronaviruses (HCoVs) have been identified in the late 1960s, involvingHCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43, severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus, and SARS-CoV-2 (the newly detected HCoV in late 2019) (Owusu*et al*., 2021).Coronavirus (CoV) is a member of the

* Corresponding author. e-mail: meqdam@just.edu.jo.

Coronaviridae family including alpha (α), beta (β), gamma (γ), and delta (δ), with a single-stranded positive-sense RNA genome, and infects humans and a diversity of animals. HCoVs are widely known as respiratory pathogens. In 1975, some researchers noticed coronavirus-like particles (CVLPs) in fecal specimens collected from patients with diarrhea (Payne *et al* 1986). Following that, human coronavirus was detected in children with AGE and in necrotizing enterocolitis in babies (Osborne *et al*., 2015, Xiong*et al*., 2020).

The current study aimed to explore the presence of HCoVsin patients with AGE and to examine the relationship between AGE and the clinical data obtained from all age groups of patients.

2. Materials and Methods

2.1. Patients and Specimens

The Institutional Review Board Committee of Jordan University of Science and Technology, Jordan approved this study (No. 13/124/2019). The fecal specimens were collected from four hospitals in Northern Jordan; King Abdullah University Hospital, Prince Rashid Ben Al-Hasan Military Hospital, Princess Basma Teaching Hospital, and Princess Rahma Teaching Hospital in Northern Jordan. A convenience sampling technique was used to collect 401fecal specimens, during the period from Sep. 2019 until Aug. 2020. This study includes patients with AGE symptoms. Clinical profiles containing significant information such as gender, age, stool properties, symptoms, etc., were gathered in a particular form. The fecal suspension was prepared for each sample by adding 0.5 ml of stool to 450 µl of phosphate buffer saline in a 1.5 ml sterile Eppendorf tube. After that, the suspension was vortexed and centrifuged for 5 min at 3000 rpm for clarification purposes. The supernatant for each sample was transferred to a new sterile Eppendorf tube and preserved at -20°C until processed.

2.2. RNA Extraction

The human coronavirus RNAwas extracted from the stool supernatant through the DNA/RNA Path Miniprep (Zymo USA), as instructed by the manufacturer. All extraction steps were carried out at room temperature (unless specified) and centrifuged for 30 seconds at 10,000 rpm.

2.3. Reverse Transcription (RT)

To synthesize the cDNA, the protocol described by Kang *et al*. (2009) was used to reverse transcribe the extracted RNA using randomprimers by Maxime RT PreMix-iNtRON kit (South Korea).Using the Thermal Cycler TC9610-230 (Mayfield Avenue Edison, USA), the cDNA synthesis reaction was performed as follows: 45°C for 60 min, followed by inactivating the RTase at 95°C for 5 min. As an internal expression control, the β-actin gene was used.

2.4. Polymerase Chain Reaction (PCR)

The primers used to detect the presence of specific genes in HCoVs were in Table 1. As described by Gouvea *et al*. (1990), the PCR reaction was carried out using the PCR master mix solution of i-TaqTM -iNtRON kit (South Korea).GeneBank ® tool was used to design the positive control and generated by GeneScript® biotech company (New Jersey, USA).Positive control and a mixture without a cDNA template as negative control were added in each run.

Table 1. Oligonucleotide primers used for detection of HCoVs.

Virus	Primer	Target region	Sequence (5′ to 3′)	Size	Ref.
HCoV- OC43	N gene	O1 Forward	CCCAAGCAAACTGCTACCTCTCAG	309bp	(Vabret *et al*., 2005)
		O3 Reverse	GTAGACTCCGTCAATATCGGTGCC		
HCoV- 229E	N gene	E1 Forward	AGGCGCAAGAATTCAGAACCAGAG	309bp	(Vabret *et al*., 2001)
		E3 Reverse	AGCAGGACTCTGATTACGAGAAAG		
HCoV- KU1	1B gene	Forward	GGTTGGGATTATCCTAAATGTGA	440bp	(Esper*et al* ., 2006)
		Reverse	CCATCATCACTCAAAATCATCATA		
HCoV-NL63	S gene	Forward	ACCGCTGTTAATGAGTCTAGATATG	523bp	(Vabret *et al*., 2005)

2.5. Gel Electrophoresis

Gel electrophoresis was performed as described by Gouvea *et al*. (1990).A volume of 5 µl of the amplified PCR products for each specimenwas analyzed using a1.5 % agarose in 100 ml of 1 x tris-borate ethylenediaminetetraacetic acid buffer and stained with 0.5 µg/ml ethidium bromide (Fisher Scientific, UK) to visualize the amplified product. The electrophoresis was performed at 120 volts at room temperature for 1 hour and the bands were imaged under a Gel Documentation System.

2.6. Statistical Analysis

The package of Social Science (SPSS) software (IBM, USA) version 1.0.0.1447 and Chi-square (X^2) test was used for statistical analysis and comparison of groups.

3. Results

The fecal specimens were analyzed by PCR to detect 4 different strains of HCoVs(HCoV 229E, HCoV HKU1, HCoV NL63, and HCoV OC43). Out of the 401 fecal

specimens,10.5% were found positive for at least one HCoV strain. HCoV 229E was the most detected virus (6.0%),

while 1.0% of cases were detected with HCoV HKU1 and HCoV. OC43 was not detected in any sample (Table 2). Figures 1-3, represent gel electrophoresis for PCR amplified products of the HCoVs detected strains. HCoVs detection among gender was demonstrated in Table 3, without significant associations (*P*> 0.05)between males and females.

Table 2: HCoVsdistribution in 401 fecal specimens

Pathogen	No. (%)
HCoV 229E	24 (6.0%)
HCoV NL63	14 (3.5%)
HCoV HKU1	4 (1.0%)
Total	42 (10.5%)

Fig. 4 demonstrated the number of positive results of 3 different viruses (HCoV 229E, HCoV NL63, and HCoV HKU1) stratified by age. Of the 401 fecal specimens, 104 (26.0%) and 60 (15.0%) specimens were obtained from children younger than 1 and equal to or more than 60 years of age, respectively. The positive rate of HCoV 229E was distributed among the age groups, 16.7 % for those aged 0-4 age group, and 14.3% for those aged 60 years or older, whereas the rate of HCoV NL63 was 14.3 % for those 0-4 age groups. The HCoV HKU1 was found only in two age groups with a percentage of 4.8% in each of the 10-19 age group and for those aged 60 years or older.

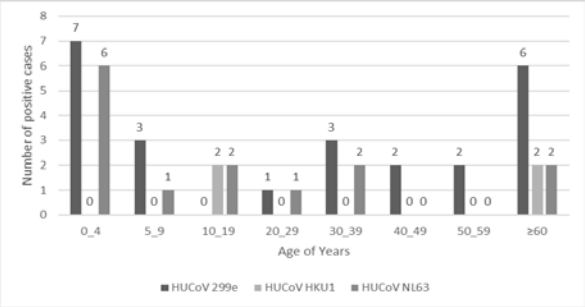

Figure 1: Distribution of human coronaviruses by patients age groups.

Table 3: The association between human coronaviruses infections and gender

Gender	HCoV 229E +ve No. (%)	HCoV 229E –ve No. (%)	HCoV HKU1 +ve No. (%)	HCoV HKU1 –ve No. (%)	HCoV NL63 +ve No. (%)	HCoV NL63 –ve No. (%)
Male	15(62.5)	211(56.1)	2 (50.0)	224 (56.6)	9 (64.3)	218 (56.3)
Female	9 (37.5)	165 (43.9)	2(50.0)	172 (43.4)	5 (35.7)	169 (43.7)
Total	24 (6)	376 (94)	4(1)	396(99)	14(3.5)	387(96.5)
P value	0.54		0.79		0.55	

The HCoVs detection varied in the four seasons (Table 4). The HCoV 229E was detected in the four seasons; winter (37.5%), autumn (29.2%, summer (25.0%), and spring were detected in two patients (8.3%), without significant associations between the HCoV 229E and seasons (*P* =0.51). A significant association was seen between the seasons and HCoV NL63 (*P* = 0.006), without any detection in spring.

Table 4: The association between human coronaviruses infection and seasons.

Season (Months)	HCoV 229E +ve No. (%)	HCoV 229E –ve No. (%)	HCoV HKU1 +ve No. (%)	HCoV HKU1 –ve No. (%)	HCoV NL63 +ve No. (%)	HCoV NL63 –ve No. (%)
Autumn	7(16.7)	119(31.6)	2 (4.7)	125 (31.6)	3 (7.2)	124 (32.0)
Winter	9(21.5)	92(24.5)	0 (0.0)	101 (25.5)	9 (21.5)#	92(23.8)
Spring	2(4.7)	35(9.3)	0 (0.0)	37(9.3)	0 (0.0)	37(9.2)
Summer	6(14.3)	130(34.6)	2 (4.7)	133(33.6)	2 (4.7)	134(34.6)
P value	0.51		0.55		0.006#	

Statistically significant at *P* =0.006

The HCoVs strains detection relationship with respiratory tract disease was demonstrated in Table 5. The HCoV 229E was detected in 2 patients with respiratory tract infection (8.3%), without significant associations (*P*=0.92).

Table 5. The association between human coronaviruses infection and respiratory tract disease.

Respiratory tract disease	HCoV 229E No. (%)	HCoV HKU1 No. (%)	HCoV NL63 No. (%)
Yes	2(8.3)	0(0.0)	0(0.0)
No	22(52.3)	4(9.6)	14(33.4)
Total No. (%)	24(57.0)	4(9.6)	14(33.4)
P value	0.92	0.72	0.27

Table 6 demonstrated the HCoVs relationship with clinical observations. A significant association was seen between the presence of HCoV 229E and fever (P = 0.04) and between HCoV HKU1 and weakness (P = 0.04). The range of symptoms duration was 2-3 days. In relation to the antibiotics treatment, 38.1% (16 out of 42) of infected patients were under antibiotics treatment.

Thirty fecal specimens were collected as control from healthy individuals to detect the presence of HCoV 229E, HCoV HKU1, HCoV NL63, and HCoV OC43 strains). Results showed that the HCoVs strains were not detected in healthy individuals.

Table 6: The association between HCoVsinfection and clinical observations

Observation	HCoV 229E+ve No. (%)	HCoV 229E –ve No. (%)	*P* value	HCoV HKU1 +ve No. (%)	HCoV HKU1 –ve No. (%)	*P* value	HCoV NL63 +ve No. (%)	HCoV NL63 –ve No. (%)	*P*value
Clinical diagnosis									
Dehydration	0(0.0)	6(1.6)	0.53	0(0.0)	6(1.5)	0.80	0(0.0)	6(1.5)	0.63
No dehydration	24(100)	371(98.4)		4(0.0)	390(98.5)		14(100)	381(98.5)	
Weakness	13(54.1)	167(44.3)	0.35	4(100)	177(44.5)	0.04#	8(57.1)	173(44.7)	0.35
No weakness	11(45.9)	210(55.7)		0(0.0)	220(55.5)		6(42.9)	214(55.3)	
Vomiting	3(12.5)	85(22.5)	0.24	0(0.0)	88(22.1)	0.28	2(14.2)	86(22.2)	0.48
No vomiting	21(87.5)	292(77.5)		4(100)	308(77.9)		12(85.8)	301(77.8)	
Fever	2(8.3)	102(27)	0.04#	0(0.0)	105(26.4)	0.23	5(35.7)	99(17.4)	0.39
No fever	22(91.7)	274(73)		4(100)	292(73.6)		9(64.3)	288(82.6)	
Abdominal pain	7(29.1)	135(35.8)	0.50	0(0.0)	143(36)	0.13	2(14.2)	140(37)	0.09
No Abdominal Pain	17(70.9)	242(64.2)		4(100)	254(64)		12(85.8)	247(63)	
Duration of Symptoms (days)									
1	5(20.8)	124(33)	0.28	3(75)	126(31.7)	0.17	6(42.8)	124(95.4)	0.64
2-3	17(70.9)	204(54)		1(25)	220(55.8)		7(50)	214(96.8)	
> 4	2(8.3)	48(13)		0(0)	50(12.5)		1(7.2)	49(98.0)	
Antibiotics received									
Yes	7(29.1)	142(37.6)	0.39	1(25)	148(37.2)	0.61	8(57)	141(37.2)	0.11
No	17(70.8)	234(62.3)		3(75)	248(62.8)		6(43)	246(62.8)	

Significant associations were seen between the presence of HCoV 229E and fever (P = 0.04) and between HCoV HKU1 and weakness (P = 0.04)

4. Discussion

Acute gastroenteritis was the main cause of mortality in children globally in the last two decades, with 10%of deaths in hospitalized children (Wang *et al*., 2016). In 2015, more than 1.3 million deaths due to diarrhea illness were reported including 146000 deaths among children <5 years old (Wang *et al*., 2016). The literature review showed that this study was the first study in Jordan that detected and associated HCoVs strains with AGE among all age groups of patients.

In this study, we collected fecal specimens from AGE patients to screen for the presence of HCoV 229E, HCoV HKU1, HCoV NL63, and HCoV OC43 strain as possible causes of AGE.

Human coronaviruses have already been considered pathogens infecting the respiratory tract since their discovery. However, the detection of HCoVs strains in fecal specimens was recorded in the United Kingdom since 1975 using electron microscope. After that, it had been detected in fecal specimens of AGE in many studies but with a very low prevalence rate. In a Slovenia study (Jevsnik *et al*., 2016), HCoV particles were detected in 8.7% of the tested specimens. Another study in Saudi Arabia (Kheyami *et al*., 2010) showed a 6% prevalence of HCoVs in AGE patients. A Finnish study (Risku *et al*., 2010) using molecular methods showed a 2.5% prevalence rate of HCoVs and distributed as follows: 45.5% HCoV-OC43, 27.3% HCoV-HKU1 and 18.2% HCoV-NL63. In the current study, HCoVs were detected in 10.5% of the tested specimens, and distributed as follows: 57.2 % HCoV-229E, 33.3 % HCoV-NL63, and 9.5 % HCoV-HKU1. A study from Ghana (Owusu *et al*., 2021) also reported that HCoV-229E was the most isolated strain. The HCoV-OC43 virus was not detected in the tested specimens. However, the detection rate of each strain of HCoVs varied in different regions, and the detection

of the predominant types of HCoVs associated with AGE is still unclear and needs further study.

The age distribution of HCoVs strains in this study was mostly in patients in the age groups below 5 years and equal to or above 60 years old, 31 % and 23.8%, respectively. Our results were in part similar to the result in a previous study that showed that the range of children age infected with HCoVs was 42 months (Kheyami *et al*., 2010). Another study showed that the age group affected was children less than 2 years old (Risku *et al*., 2010). No significant association was found between HCoVs infection and gender (P >0.05). The percentage of males to females was similar to previous studies (Risku *et al*., 2010).

Most cases of HCoVs were detected in the winter season (42.9%), with a significant association recorded with HCoV NL63 (P =0.006) and the lowest in the spring season (4.8%). Compared with a previous study, Risku *et al*. (2010) recorded the highest percentage of cases in both winter and spring each with 45.4%, which may be attributed to the low sample collection due to COVID-19pandemic. Paloniemi *et al*. (2015) detected HCoVs in both fecal specimens and nasopharyngeal swaps with a higher viral load in patients associated with AGE compared with respiratory tract disease, which may indicate that the source of the virus in fecal specimens is the respiratory tract.

In this study, the detected HCoVs appeared as the significant pathogens and were supported by the fact that thirty specimens collected from healthy individuals were found negative for HCoVs.

Due to a lack of published data concerning the clinical observations of AGE patients infected with HCoVs to compare with, in this study we presented the clinical observations from patients as follows: all the detected cases have a weakness; HCoV HKU1 recorded 100% weakness with significant association (P = 0.04). HCoV NL63 and HCoV 229E recorded similar percentages, 57.1%, and 54.1%, respectively. Vomiting was shown at 14.2% and 12.2% in HCoV NL63 and HCoV 339E, respectively. Also, fever recorded a significant association with HCoV 229E (P = 0.04) which represented 8.3% and HCoV NL63 recorded a higher rate in 53.7% of patients with fever. A significant association between fever and infection with human coronaviruses was also reported by Wen *et al*., 2022. The percentages of patients suffering from abdominal pain were 29.1% and 14.2% of the HCoV 339E and HCoV NL63. There were no positive cases recorded for HCoVs infection with dehydration. The duration of symptoms varies between the HCoVs strains and patients with AGE; HCoV 229E and HCoV NL63 recorded the highest percentages of duration as 2-3 days with 70% and 50%, respectively, while HCoV HKU1 recorded 75% for the one-day duration. Patients infected with HCoVs and under antibiotic treatment were 38.1%, which indicates the need for a laboratory test to diagnose viral AGE and minimize the use of unnecessary antibiotics.

5. Conclusions

The study provided data about the epidemiological features of human coronaviruses in patients with acute gastroenteritis with the association between AGE and the clinical data collected from patients in Northern Jordan. The provided data has demonstrated a 10.5% of specimens were found positive for at least one HCoVs with the highest percentage for HCoV-229E (57.1%) in Northern Jordan. Most infected cases (23.8%) were in the age group >60 years old. Among the positive specimens, 38.1% were under antibiotic treatment, necessitating viral diagnoses setting to enhance patient safety by reducing antibiotic overuse.

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