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Original Article

Immunological and Molecular Method for the Diagnosis of Human Bocavirus in Patients with Respiratory Infections in Mosul, Iraq

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ABSTRACT

Background: Human bocavirus (HBoV) typically affects adolescents and causes upper and lower respiratory infections. However, little is known about the clinical features of this pathogen and its implications for respiratory infections in adults. **Objectives:** To detect the HBoV effect on adults and adolescents causing respiratory diseases in the lower respiratory tract. **Materials and Methods:** Seventy throat and nasopharyngeal swabs were collected from male and female patients, aged 11–57 years, from three hospitals in Mosul City, Iraq, within 6 months in 2024. The samples were stored in a viral transport medium until indirect enzyme-linked immunosorbent assay and real-time polymerase chain reaction (RT-PCR) were performed. **Results:** Out of 70 samples, the prevalence rate of immunoglobulin (Ig)M antibodies was 37 (53%), IgG was 33 (47%), and viral DNA for HBoV was 24 (34%), respectively. The dominating age groups were above 30 years, and males were more susceptible to infection compared with females. **Conclusion:** The best diagnostic method for the detection of HBoV is RT-PCR. The present study highlights RT-PCR in diagnosing respiratory infections. This technique can be compared with other immunological methods to determine the severity of the disease.

Keywords: Bocavirus, ELISA, Fast-Track Respiratory Pathogens 33, real-time PCR, respiratory tract infections

Introduction

Human bocavirus (HBoV) belongs to the Parvoviridae family, subfamily, and genus. Random genome amplification led to the discovery of the HBoV, which, following sequence analysis, was provisionally classified as an HBoV strain. The HBoV genome phylogenetic analysis showed that it was closely related to Bovine Parvovirus and Minute Virus of Canines, after which it was named.[1]

Parvoviridae infections can contaminate different hosts. Although the classification of related clinical problems is still in its early stages, it appears this virus is linked to systemic infections and respiratory tract diseases.[1] It was discovered that the virus frequently showed up in respiratory secretions taken from young children, with prevalence rates ranging from 1% to 19%.[2] HBoV is a small, nonenveloped virus having icosahedral symmetry and a 5 kb single-stranded deoxyribonucleic acid (ssDNA) genome. Two critical structural proteins, VP1 and VP2, and two nonstructural proteins, NS1 and nuclear phosphoprotein 1, are encoded by the genome (NP1). Based on the nucleotide (nt) dissimilarity of the VP1 capsid region, HBoVs fall into four species: HBoV1, HBoV2, HBoV3, and HBoV4.[2,3] They have a ssDNA genome and create minor capsids. It has three proteins with known functions encoded by the viral genome: the nonstructural protein NS1 and the two-capsid proteins VP1 and VP2.[4] HBoV is found more regularly in clinical samples from patients with respiratory sicknesses. Though HBoV2, HBoV3, and HBoV4 are more commonly associated with gastrointestinal diseases, their particular

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part as enteropathogens is yet obscure.[5] HBoV1 was found in pediatric respiratory examples in 2005, and it was the first human-pathogenic parvovirus. Ever since several studies have been conducted to investigate its association with acute respiratory tract disease. Bronchitis, pneumonia, asthma, and the common cold are the most frequent clinical signs of intense HBoV1 contamination.[6]

HBoV 1–4 has been studied for its pathogenicity in patients with respiratory diseases, gastroenteritis, heart diseases, meningitis/encephalitis, fetal hydrops or mortality, cancer, and transplant recipients/immunocompromised.[7] The core is where the parvovirus replication and assembly occur, which is dependent on the host's cellular activity. The genome replication mechanism of the viral family is fascinating. The fastener structure at the 3′ end works as a self-primer, causing plus-sense DNA to be synthesized and double-stranded DNA (dsDNA) to be delivered.[8]

The fastener shape is a framework to decipher extra minus-sense strands from DS DNA. Consistent with the current evidence, the amplified strand folds back on itself to create a tetrameric shape, which is, along these lines, divided into two plus-sense and two minus-sense DNA strands.[9] During replication within the core, the connection to receptors triggers clathrin-mediated endocytosis of the virion into the host cells. The virion moves to the core after entering the cytoplasm. The virus's ssDNA genome enters the core. Cellular proteins change from ssDNA to dsDNA when the cell enters the S stage.

The dsDNA translation occurs, and viral mRNAs deciphered into viral proteins at that point. For replication, the rolling hairpin mechanism is utilized. The NS1 endonuclease covalently connects to the 5′ genomic end. These newly made ssDNA molecules can either be converted to dsDNA and be used as a template for transcription and replication, or they can be encapsidated and released by cell lysis.[10,11] The study aims to investigate the effects of HBoV, which causes lower respiratory tract diseases, on adults and adolescents.

Materials and Methods

Collection of samples

Seventy samples from the nasopharyngeal and throat swabs were taken from respiratory tract-infected patients from three hospitals in Mosul city (Al-Shifaa Hospital for Infectious Diseases, Al-Salam Teaching Hospital, and Advisory Clinic for Chest and Respiratory Diseases at Al-Faisalia in Mosul City) within 6 months in 2024. The swabs were kept in a viral transport medium and immediately transported to the laboratory, where they were kept in a deep freeze (−70°C) until they were used.

Enzyme-linked immunosorbent assay (ELISA)-based HBoV-immunoglobulin (Ig)M and IgG antibodies detection

The samples from nasopharyngeal and throat swabs were analyzed for HBoV-IgM and IgG antibodies using an ELISA kit following the manufacturer's instructions (SunLong Biotech Co., Ltd, HangZhou, China).

Extraction of viral DNA and real-time polymerase chain reaction (PCR) process

All samples were retested with real-time PCR (RT-PCR). Viral DNA was extracted using the QIAGEN QIAamp® Viral DNA Mini Kit, according to the manufacturer's recommendations (Germany). PCR techniques have substantially advanced since quantitative RT-PCR was developed to amplify viral DNA. Real-time methods are used Fast-Track Diagnostics Respiratory Pathogens 33 (FTD-33) kit from Luxembourg. The FTD test was the only way to identify *Bocavirus* from the rest of the Boca Parvovirus family because most are identified as *Bocavirus*. Specific pathogen sequences in the process are indicated by a spike in fluorescence seen from the related dual-labeled probe, which the Real-Time Thermocycler displays as a cycle threshold value (C_t). During the lysis buffer step of the extraction process, the Equine Arteritis Virus was used as an internal control and a negative control utilizing an Applied Biosystems Real-time PCR7500, Germany.[12,13]

Results

Seventy throat and nasopharyngeal swab samples from respiratory tract-infected patients of both sexes and varied ages were subjected to indirect ELISA and RT-PCR assays to determine the presence of IgM, IgG antibodies, and viral DNA for HBoV, respectively.

Detection of HBoV-IgM and IgG antibodies

As indicated in Table 1 and 2, indirect ELISA findings reveal that the prevalence of HBoV-IgM and IgG

Table 1: Number and percentage of HBoV Using ELISA for IgM detection according to age and sex

Age (years)		Number of patients with HBoV infection by HBoV-IgM detection	
		Males	Females
Under 20	5	3	2
20–29	10	6	4
30–39	13	7	6
Above 40	9	5	4
Total	37	21	16
		57%	43%
Total out of 70 = 53%			

antibodies is 37 (53%) and 33 (47%) out of 70 samples, respectively. The prevalence of the virus is higher in males than in females, reaching 57% for anti-HBoV-IgM and 67% for anti-HBoV-IgG. Furthermore, the 30–39 years age range exhibits the most significant number of infections, 13 out of 37 positive samples and 12 out of 33 positive samples) for HBoV-IgM and IgG antibodies, respectively.

Table 2: Number and percentage of HBoV using ELISA for IgG detection according to age and sex

Age (years)		Number of patients with HBoV infection by HBoV-IgM detection	
		Males	Females
Under 20	5	4	1
20–29	9	7	2
30–39	12	6	6
Above 40	7	5	2
Total	33	22	11
		67%	33%
Total out of 70 = 47%			

Table 3: Number and percentage of HBoV using real-time (RT-PCR) according to age and sex

Age (years)		Number of patients with HBoV infection by Rt-PCR	
		Males	Females
Under 20	3	2	1
20–29	7	5	2
30–39	9	4	5
Above 40	5	3	2
Total	24	14	10
		58%	42%
Total out of 70 = 34%			

Detection of HBoV- DNA nucleic acid

The ages of the male and female patients in the present study ranged from 11 to 57 years. The mean age of HBoV respiratory tract infection of all patients was 32.16 ± 11.82, 31.28 ± 12.56 and 33.4 ± 11.23, respectively.

Approximately 24 swabs (34%) out of 70 samples tested positive for HBoV infection in RT-PCR [Table 3]. The amplification reaction appeared bocavirus-specific at a high C_t value (30.56). However, the mean C_t value of HBoV infection for all the patients was 34.00 ± 2.20, 34.05 ± 2.17, and 33.94 ± 2.37, respectively [Figure 1].

Discussion

HBoV was initially identified by isolating the respiratory secretions of adolescents suffering from respiratory illnesses in 2005.[14] Clinical symptoms alone are not sufficient to diagnose HBoV infection. Several laboratory techniques can help diagnose it, including serology, antigen detection, PCR, and electron microscopy[15-17] Most of these techniques are not suitable for routine diagnosis. However, serology can determine if the infection is acute or past and can be used to corroborate the results of other techniques.[18] Studies conducted in Iraq and other parts of the world have mainly focused on the HBoV infection in newborns and adolescents, perhaps because this age group is more susceptible to infection compared to adults.[17,19-21] Furthermore, other studies have reported that most HBoV infections occur in adults with immunodeficiency.[22,23] The current results revealed that infected patients were sufficiently immune and susceptible to infection with this virus, especially in the 30–39 years age group. The prevalence of HBoV-IgM antibodies was 37 (53%). These results are greater than those of adolescents with HBoV infection reported in other studies. In Iraq, the rate was 16.5% in Baghdad, Diyala/Iraq, and 8.8% in Egypt at 13%, as well as in Germany and Finland.[8,17,19,24,25] The

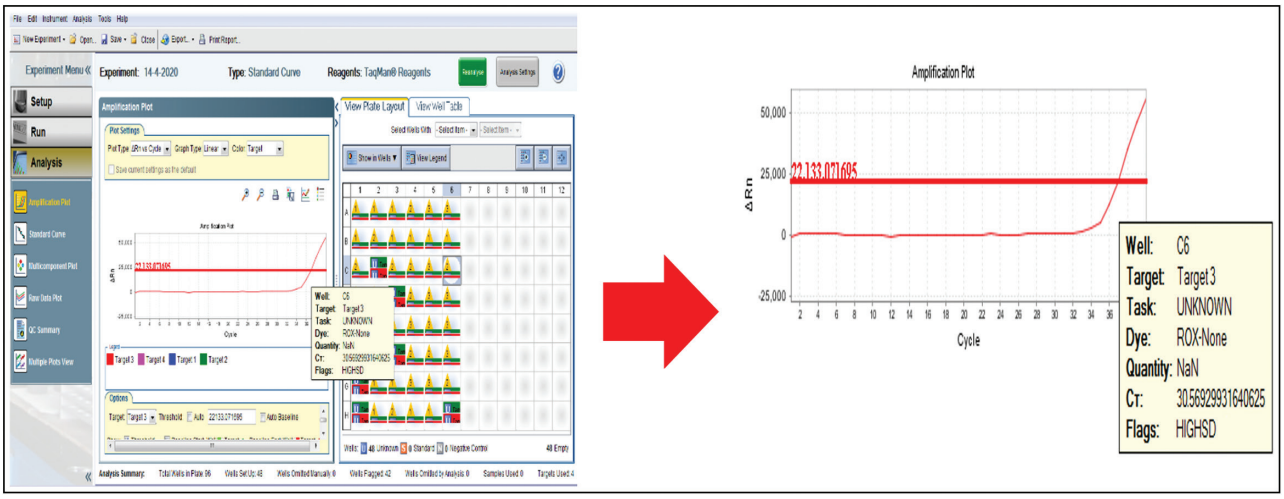

Figure 1: An amplification plot showing the quantity of fluorescence acquired in each amplification cycle. C_t (30.56) represents HBoV infection

positive result for the IgG antibodies was 33 (47%). Our results are in agreement with those of another study done in Finland, where IgG antibodies were detected in 43% of pediatric patients, compared with 35% and 31% in previous studies.[19,26,27] In 2008, serological research revealed systemic respiratory tract infections caused by HBoV1 triggered B-cell immune responses.[28] Since then, measuring HBoV1-specific immunoglobulin IgM and IgG have been utilized to diagnose acute HBoV1 infection.

Data from the RT-PCR investigation showed a good result at high (C_t = 30.56). The 24 (34%) were positive for HBOV. In Iraq, the prevalence of the virus worldwide was 38.8%, 24.62%, and 9.9% using RT-PCR.[17,19,29] This difference might be attributed to a variety of factors, including sample-related processes, kind of sample, method of collection, preservation, duration, age differences, and cultural, social, and economic levels, in addition to variations in the methods employed for viral detection.[19,30]

Regarding age and sex, our study of both techniques indicates that the 30- to 39-year-old age range is most vulnerable to HBoV infection and that the male infection rate is higher than that of females. Numerous studies indicate that males are more susceptible to respiratory viruses than females due to hormonal, physiological, and genetic variations. Females have a higher immune response than men, enabling them to eradicate infections rapidly.[31-35] HBoV infection, even though it has distinct epidemiological features.[36] In adolescents with respiratory problems, HBoV is commonly found. It might cause life-threatening hypovolemic shock due to diarrhea in an immunocompromised child with disseminated HBoV infection.[37] There is no significant difference in the HBoV infection rate in Iraq compared with those in the neighboring countries, except with nasal discharge and wheezing.[3]

Asymptomatic individuals with prolonged HBoV shedding and HBoV detections may be linked to HBoV persistence, at least in a yet-to-be-defined subset of patients. Symptomatic individuals, on the other hand, have initial infection or reinfection.[5] Rhinovirus, Bocavirus, and adenovirus cases do not follow a seasonal pattern; they are seen throughout the year.[38,39] Exposure to smoking is thought to increase epithelial susceptibility to viral infection by raising the number of receptors in youngsters whose relatives smoke. Smoking is a significant contributor to the viral infection of the respiratory tract, increasing morbidity and mortality and initiating latent infection.[13] HBoV DNA has been found in lung and colorectal tumors, suggesting that the virus may persist in some organs. HBoV persistence is a possibility and can cause long-term illness in some patients.[5] Acute respiratory infections (ARI) in adolescence caused by HBoV are thought to be responsible for 5%–15% of all ARI cases in adolescents.[40] Cough is the most prevalent symptom across all illnesses in patients with respiratory viruses. Patients with bocavirus and parainfluenza 2–4 have the longest duration of symptoms (7 days). Women with bocavirus have the highest severity score of 22, which ranges from 6 to 50.[41]

Conclusion

HBoV can typically cause upper and lower respiratory infections in adults and adolescents. The best diagnostic method is RT-PCR. The present study highlights RT-PCR in diagnosing respiratory infections. This technique can be compared with other immunological methods to determine the severity of the disease.

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Conflict of interest

There are no conflicts of interest.

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