Human Bocavirus among Viral Causes of Pediatric Respiratory Tract Infections at Benha University Hospital

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ABSTRACT

Background/Aim: Human bocavirus (HBoV) is a recently discovered parvovirus associated with acute respiratory tract infections in children. The aim of this study was to define the epidemiological profile and the clinical characteristics associated with HBoV infection in a population of children hospitalized with respiratory tract infections at Benha University Hospital, especially in terms of HBoV load.

Methods: 200 nasopharyngeal aspirates were collected and analyzed at the period from September 2010 to July 2011 from children with respiratory manifestations, their ages ranged from 1 month to 12 years. All samples were tested for HBoV DNA by quantitative real time PCR and tested by Ag detection immunofluorescence test for 8 of the most important viruses encountered in lower respiratory tract infections in infants & children namely, Adenovirus(ADV), Influenza A and B (Flu-A and B), Parainfluenza 1,2 and3 (PIV 1–3), Human metapneumovirus (HMPV) and Respiratory syncytial virus (RSV).

Results: Our results revealed that HBoV was the most prevalent virus 20/200 (10%) followed by RSV (8%) and HMPV (4%). 15/20 (75%) of HBoV+ samples were in co-infection with one of the tested viral agents and the majority of the co-infections, 10/15 (66.7%) were HBoV-RSV co-infection. The mean age of HBoV+ cases was 19.1 months and the majority 14/20 (70%) were under 2 years old. HBoV+ cases were concentrated in the winter season. No significant differences were found in term of age , gender or frequencies of respiratory manifestations between patients with sole HBoV infection and patients with co-infection with other viruses. The median viral load in patients with sole HBoV infection (196x10^5) was significantly higher than those who had co-infection with other respiratory viruses (0.033x10^5) (p=0.006). The manifestations observed in patients with serious lower respiratory tract infections, like tachypnea, dyspnea and cyanosis, were significantly presented more frequently in children with high HBoV loads than those with low HBoV load. On conclusion: HBoV is one of the most prevalent respiratory viruses and plays an important role in respiratory illness in children.

INTRODUCTION

Acute respiratory tract infections (ARTIs) are a leading cause of hospitalization and illness, in infants and young children and they represent the leading killers of children under 5 years of age, resulting in around 1.9 million of deaths worldwide annually. Respiratory syncytial virus (RSV), human metapneumovirus (HMPV), influenza viruses, human coronaviruses, rhinoviruses, and adenoviruses are some of the most important viral agents for this group of patients[1,2].

Over the last decade, modern molecular techniques have led to the discovery of several previously unknown respiratory tract viruses, including HMPV, severe acute respiratory syndrome (SARS) coronavirus, two new human coronavirus types, (HCoV-NL63) and (HCoV-HKU1), and two new human polyomaviruses[3].

In 2005, Allander et al.[4] reported a previously undescribed human parvovirus in respiratory secretions of children with respiratory tract diseases in Sweden. Phylogenetic analysis showed that this virus belonged to the genus Bocavirus (subfamily, Parvovirinae; family, Parvoviridae) and was most closely related to bovine parvovirus (BP) and minute virus of canines (MVC), and thus the virus was named “human bocavirus” (HBoV). HBoV includes small non-enveloped, icosahedral viruses with 5.3 kb single-stranded DNA genome. Recent studies conducted in different countries have shown that HBoV is found in 1.5 - 19% of children with respiratory diseases. HBoV has been observed to be associated with a broad spectrum of both upper and lower respiratory tract diseases, more frequently related to lower respiratory diseases[5].

HBoV infection has recently attracted increasing attention all over the world and increasing evidences are emerging to support its role as an etiologic agent in upper and lower respiratory tract infection and gastrointestinal illness throughout the world[6]. However, the incidence and clinical presentation of this infection varies widely, and often involves co-
infection with other potential pathogens. Such characteristics have led to debate over the role of HBoV as a true pathogen and make it difficult to evaluate the etiological role of HBoV in respiratory disease.[9]

Although it was found that HBoV could be cultured in differentiated human airway epithelial cells,[8], routine viral culturing of HBoV remains difficult. Real-time PCR has been used to estimate viral load and its usefulness has been proved as an indicator of the degree of active viral infection.[9]

PATIENTS & METHODS

This study was held in Microbiology and Immunology Department, Faculty of Medicine, Benha University, in the period from September 2010 to July 2011. The study was conducted on 200 pediatric patients attending the Pediatric Inpatient and Outpatient Clinic of Benha University Hospital, their ages ranged from 1 month to 12 years (mean age 32.1 months). They were 112 males and 88 females.

After informed written consent was obtained from parents or guardians, all patients were subjected to full history taking and thorough clinical examination. Patients were suffering from different specific and non-specific upper and lower respiratory tract manifestations such as cough, rhinorrhea, fever, wheezing, tachypnea, dyspnea and cyanosis.

Sample collection:

A total of 200 nasopharyngeal aspirates were collected by mucous extractor and transferred to each vial of the universal transport medium MicroTest Transport System M4 (Oxoid) which contains gelatin, vancomycin, amphotericin B and colistin. The 200 aspiration samples were stored in aliquots at -75°C until further processing.

After thawing, the extracted DNA was kept at -75°C until viral Ag detection, nucleic acid extraction and PCR proceeded.

Direct Ag detection of respiratory viruses:

This test was done to detect viral Ag directly from nasopharyngeal aspirates of 8 of the most important viruses encountered in lower respiratory infections in infants & children namely, Adenovirus(ADV), Influenza A and B (Flu-A and B), Para influenza 1,2 and3 (PIV 1–3), Human metapnuemovirus (HMPV) and Respiratory syncytial virus (RSV) using direct fluorescent assay.

Two ml of specimen in transport medium were centrifuged for 10 min at 1000xg to re-pellet cells then supernatant was removed and 500 µl of PBS were added to re-suspend the cell pellet for testing by D3 Ultra Duet for direct specimen testing of respiratory viruses catalog No 01-010008.v2 (Diagnostic HYBRIDS, German).

One 8 wells slide was labeled for each specimen, then 20 µl of cell pellet were transferred using transfer pipette to each well after that the slide was left to air dry for 60 min. then one drop of each individual D3 ultra duct monoclonal antibodies were added to the wells according to the testing orders as follows well 1=ADV, Well 2=HMPV, well 3=Flu-A, well 4=Flu-B, well 5=PIV-1, well 6=PIV-2, well 7=PIV-3 and well 8=RSV. Slides then incubated at 37°C for 15 min. and then gently washed by PBS. After that, one drop of mounting medium was added to each well and cover slip was placed above, finally slides were examined by fluorescent microscope at X 200 magnifications.

Real-time PCR for HBoV:

QIAamp DNA Mini and Blood Mini kit (QIAGEN, Hilden, Germany) was used to extract viral DNA according to manufacture instructions then the extracted DNA was kept at –75°C until further processing.

To detect and quantify the viral loads of HBoV, a universal Taqman real-time PCR that can detect HBoV1–4 was performed. The primers/probe of the Taqman real-time PCR were designed to target the conserved regions of the viral protein (VP) 1/2 gene segment of HBoV1–4, amplifying a 113-bp fragment. The sequence of the forward primer was 5′-TGGMATTATTTGGMTCMAGTTT-3′, the reverse primer was 5′-CACCCTTTAATTTGAGTTDGCA-3′, and that of the probe was 5′-AAGCGCGCCGTGGCCTCTGCTCT-BHQ-1–3′, corresponding to the HBoV1 st1 strain (GenBank accession no. DQ000495) nt 3316–3328, corresponding to the HBoV1 st1 strain (GenBank accession no. DQ000495) nt 3316–3328.

The 20-µL amplification reaction contained 5 µL of sample DNA, 10 µL of TaqMan universal PCR master mix which contains 2X reaction buffer, 0.025 U/µL Taq Polymerase, 5 mM MgCl2, dNTP Mix (200µM each dNTP) (PrimerDesign Ltd, USA), 0.1 µL of bovine serum albumin (20 mg/mL), 1 µL of HBoV Primer/Probe mix300 nm ol/L and 4 µL RNase/DNase free water (PrimerDesign Ltd, USA)
Preparation of standard curve dilution series:
900 µl of RNAse/DNAse free water were Pipetted into 5 tubes and labelled from 2 to 6 then 100 µl of Positive Control Template (RED) were Pipetted into tube 1 after that it was Vortexed thoroughly and pipette tip was changed and 100 µl were pipetted from tube 1 into tube 2 then it was vortexed thoroughly lastly this step was repeated to complete the dilution series so as the tubes from 1 to 6 contains 2 × 10^5 copies/µl, 2 × 10^4 copies/µl, 2 × 10^3 copies/µl, 2 × 10^2 copies/µl, 20 copies/µl and 2 copies/µl respectively.
NB: For negative control wells 5 µl of RNAse/DNAse free water were used & the final volume in each well was 20 µl.

Amplification Protocol:
Sequencing and amplification were performed using ABI 7900 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) with the following instrument settings: 95°C for 10 min, 50 cycles of 95°C for 10 seconds, and 60°C for 1 min. Samples and the PCR mixtures were prepared under laminar flow hoods in separate rooms to prevent contamination especially with positive control.

RESULTS
The result of our study revealed that 59/200 cases (29.5%) were infected with one or more viruses tested in this study. HBoV genome was detected in 20 out of 200 (10%) respiratory samples. So, this study found that HBoV was the most prevalent virus among the other respiratory viruses followed by RSV (8%) and HMPV (4%) (Table 1).

In our study, 15 out of 20 HBoV+ samples (75%) were in co-infection with one of the tested viral agents, while it was the sole virus in 5 patients (25%). The majority of the co-infections, 10/15 (66.7%) were HBoV-RSV co-infections (Table 2).
HBoV was detected in the age range of 7 months to 4 years with mean and median age of 19.1 and 14.5 months respectively. The majority of HBoV+ patients 14/20 (70%) were under 2 years old. HBoV+ cases were concentrated in the winter season. The male:female ratio in the HBoV-positive patients was (12:8) which did not differ significantly from that in HBoV-negative patients (100:80) (p = 0.7).

The clinical characteristics of the HBoV+ patients were summarized in (Table 3). The most frequent respiratory symptoms/signs seen in HBoV-positive patients were cough (100%) and wheezing (90%). No significant differences were found in term of age, gender or frequencies of respiratory manifestations between patients with single HBoV infection and patients with co-infection with other viruses (Table 3).

Absolute quantification of DNA by quantitative PCR revealed that the HBoV viral load in HBoV-positive patients varied very broadly, ranged from 0.0074 × 10^5 copies/mL to 39000 × 10^5 copies/mL (median= 0.0505 × 10^5). The median viral load which was found in patients with sole HBoV infection (196 × 10^5) was significantly higher than those who had co-infection with other respiratory viruses (0.033 × 10^5) (p=0.006)(Table 3).

The HBoV-positive cases were categorized in two groups: low viral load group (viral load <10^4 copies/mL, N=13) and high viral load group (viral load ≥10^4 copies/mL, N=7). The comparisons of demographic and clinical data between the two groups were shown in (Table 4). The distributions of age and gender were not significantly different between the two groups. Non-specific respiratory tract symptoms, such as fever, cough and rhinorrhea, had no significant correlation with HBoV viral load. However, the manifestations observed in patients with serious LRTIs, like tachypnea, dyspnea and cyanosis, were significantly presented more frequently in children with high HBoV viral loads than those with low HBoV load.

Table (1) Prevalence of respiratory virus infections determined by direct Ag detection.

<table>
<thead>
<tr>
<th>Respiratory virus</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADV</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>HMPV</td>
<td>8 (4%)</td>
</tr>
<tr>
<td>Flu-A</td>
<td>7 (3.5%)</td>
</tr>
<tr>
<td>Flu-B</td>
<td>5 (2.5%)</td>
</tr>
<tr>
<td>PIV-1</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>PIV-2</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>PIV-3</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>RSV</td>
<td>16 (8%)</td>
</tr>
<tr>
<td>Total</td>
<td>54 (27%)</td>
</tr>
</tbody>
</table>
Table (2) Co-pathogens of HBoV-positive patients.

<table>
<thead>
<tr>
<th>HBoV co-infection (n=15)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBoV+ RSV</td>
<td>10 (66.7%)</td>
</tr>
<tr>
<td>HBoV+ PIV</td>
<td>2 (13.3%), 2 PIV-3</td>
</tr>
<tr>
<td>HBoV+ Flu virus</td>
<td>2 (13.3%), 1 Flu-A and 1 Flu-B</td>
</tr>
<tr>
<td>HBoV+ ADV</td>
<td>1 (6.7%)</td>
</tr>
</tbody>
</table>

Table (3) Comparison of demographic and clinical data between patients with single HBoV infection and patients with co-infection with other viruses.

<table>
<thead>
<tr>
<th></th>
<th>Single HBoV infection (n = 5)</th>
<th>HBoV co-infection (n = 15)</th>
<th>Total (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age</td>
<td>12 months</td>
<td>17 months</td>
<td>14.5 months</td>
<td>0.57 NS</td>
</tr>
<tr>
<td>Sex</td>
<td>3 male : 2 female</td>
<td>9 male : 6 female</td>
<td>12 male : 8 female</td>
<td>1.0 NS</td>
</tr>
<tr>
<td>Median Viral load (x10³)</td>
<td>196</td>
<td>0.033</td>
<td>0.0505</td>
<td>0.006 S</td>
</tr>
<tr>
<td>Clinical data</td>
<td></td>
<td></td>
<td></td>
<td>---------</td>
</tr>
<tr>
<td>-Cough</td>
<td>5 (100%)</td>
<td>15 (100%)</td>
<td>20 (100%)</td>
<td>--------</td>
</tr>
<tr>
<td>-Rhinorhea</td>
<td>4 (80%)</td>
<td>11 (73.3%)</td>
<td>15 (75%)</td>
<td>0.76 NS</td>
</tr>
<tr>
<td>-Fever</td>
<td>3 (60%)</td>
<td>9 (60%)</td>
<td>12 (60%)</td>
<td>1.0 NS</td>
</tr>
<tr>
<td>-Wheezing</td>
<td>5 (100%)</td>
<td>13 (86.7%)</td>
<td>18 (90%)</td>
<td>0.38 NS</td>
</tr>
<tr>
<td>-Tachypnea</td>
<td>4 (80%)</td>
<td>9 (60%)</td>
<td>13 (65%)</td>
<td>0.41 NS</td>
</tr>
<tr>
<td>-Dyspnea</td>
<td>2 (40%)</td>
<td>6 (40%)</td>
<td>8 (40%)</td>
<td>1.0 NS</td>
</tr>
<tr>
<td>-Cyanosis</td>
<td>2 (40%)</td>
<td>5 (33.3 %)</td>
<td>7 (35%)</td>
<td>0.78 NS</td>
</tr>
</tbody>
</table>

Table (4) Demographic and clinical data of HBoV-positive patients on the basis of viral loads.

<table>
<thead>
<tr>
<th></th>
<th>Low viral load &lt;10⁴ copies/mL (n = 13)</th>
<th>High viral load ≥10⁴ copies/mL (n = 7)</th>
<th>Total (n= 20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age</td>
<td>15 months</td>
<td>14 months</td>
<td>14.5 months</td>
<td>0.91 NS</td>
</tr>
<tr>
<td>Sex</td>
<td>8 male : 5 female</td>
<td>4 male : 3 female</td>
<td>12 male : 8 female</td>
<td>0.77 NS</td>
</tr>
<tr>
<td>Clinical data</td>
<td></td>
<td></td>
<td></td>
<td>---------</td>
</tr>
<tr>
<td>-Cough</td>
<td>13 (100%)</td>
<td>7 (100%)</td>
<td>20 (100%)</td>
<td>--------</td>
</tr>
<tr>
<td>-Rhinorhea</td>
<td>9 (69.2%)</td>
<td>6 (85.7%)</td>
<td>15 (75%)</td>
<td>0.41 NS</td>
</tr>
<tr>
<td>-Fever</td>
<td>7 (53.8%)</td>
<td>5 (71.4%)</td>
<td>12 (60%)</td>
<td>0.44 NS</td>
</tr>
<tr>
<td>-Wheezing</td>
<td>11 (84.6%)</td>
<td>7 (100%)</td>
<td>18 (90%)</td>
<td>0.26 NS</td>
</tr>
<tr>
<td>-Tachypnea</td>
<td>6 (46.1%)</td>
<td>7 (100%)</td>
<td>13 (65%)</td>
<td>0.016 S</td>
</tr>
<tr>
<td>-Dyspnea</td>
<td>2 (15.4%)</td>
<td>6 (85.7%)</td>
<td>8 (40%)</td>
<td>0.002 S</td>
</tr>
<tr>
<td>-Cyanosis</td>
<td>2 (15.4%)</td>
<td>5 (71.4%)</td>
<td>7 (35%)</td>
<td>0.012 S</td>
</tr>
</tbody>
</table>

**Statistical analysis:**
The collected data were analyzed using SPSS version 16 software, categorical data were presented as number and percentage while continuous variables were expressed as mean ± SD, median and range. Chi square test, Z test and Mann Whitney U tests were used as tests of significance. The accepted level of significance in this work was stated at 0.05 for 2 sided P value.

**DISCUSSION**
Here we report the viral etiologies of ARTIs in 200 hospitalized children enrolled during a period from September 2010 to July 2011. Overall, HBoV was the most frequently detected virus, and accounted for 10% of the infections. RSV (8%) was the second most common virus detected, followed by HMPV (4%) (Table1). Many different studies indicating that RSV is the dominant cause of respiratory tract infections in children and that HBoV is the second or third most commonly detected virus[10-15]. These discrepancies with our results may be explained by the different detection methods; molecular diagnostic methods were used for the detection of other respiratory viruses in the other studies whereas, in our study, we used direct immunofluorescence assay with less sensitivity.

During the last years since its discovery, HBoV has been detected in association with respiratory infections, mostly of children, in many countries. In the present study, the overall
The detection rate of HBoV in ARTI patients was 10%, similar to rates in many other studies and within the range of the data reported by other authors who have detected the virus in 1.5 to 19% of subjects with acute respiratory illness[5,6,11,16-19]. However, Zaghloul et al.[20] in their study detected HBoV in higher prevalence rate as twenty two (22%) out of the 100 NPA specimens of the patients with respiratory manifestations were positive for HBoV by qualitative PCR. However, it should be kept in mind that our study, like most others published on HBoV, was a hospital-based surveillance, subject to selection bias. Therefore, rates of HBoV in ARTIs in the community at large could be different.

This wide range of detection of HBoV can at least partially be attributable to multiple factors, such as patient's age, season, geographic location, laboratory technique, primer sensitivity, type of sample or may be due to true variation in incidence of HBoV[17,21].

Characteristics of HBoV-positive patients in our study were also similar to previous reports[5,17,18,22-24], the male:female ratio in the HBoV-positive patients did not differ significantly from that in the HBoV-negative patients (P=0.7).

HBoV infection has been shown (by PCR of NPA samples) to be most prevalent in children 6 months to 5 years of age; adults are less affected[5,19,23,25]. In consistent with this finding, our results found that the age range of HBoV+ cases extended from 7 months to 4 years, with mean age of 19.1 months. Most HBoV+ patients (70%) were less than 2 years old.

Since frequency of HBoV infection, as confirmed by our study and many other authors[4,19,25], was found to be relatively low in children younger than 6 months, some authors proposed that maternal antibodies prevent neonatal infection by HBoV during the first 6 months[26-28]. In contrast, many other studies have detected HBoV as early as few days after birth and a seroepidemiological study also provided evidence that HBoV infection is common during early infancy suggesting an incidence of HBoV infection very early in life[15,29,30].

The high prevalence rate in young children led to the hypothesis that HBoV may be an endemic virus with high attack rates in those susceptible; one would then expect that the majority of the population would be infected during childhood[31]. In support of this hypothesis are the high seroprevalence rates against HBoV reported in the adult population[32]. Also, the lack of variation in the surface protein of HBoV suggests that HBoV infection may happen only once, with the subsequent development of life-long immunity via neutralizing antibodies[33]. This is consistent with the fact that HBoV infection occurs primarily in infants and young children.

Regarding the HBoV seasonality, seasonal peaks of HBoV infection vary among different countries and regions because of climate and other factors[34]. In our study, HBoV+ cases peaked during winter months. Similar findings have been reported[10,12,18,25,28,30,33] although some studies found HBoV cases throughout the year with spring outbreaks[37,34,35]. Many authors explain this seasonal peak of HBoV infection during winter months by that most surveys on HBoV so far analyze samples collected for diagnosis of ARTIs, which is the reason for the predominance of samples collected during winter. Other possibility is that the increased incidence of co-infections with other respiratory viruses which peaked during cooler and drier months[36]. In contrast, Bastien et al.[36] discovered that there was no obvious regular seasonal occurrence of HBoV by examining 1209 Canadian specimens collected during two continuous years.

Viral co-infections have long been recognized in ARTIs studies and the advent of highly sensitive PCR assays magnified this observation. In the particular case of HBoV, frequencies of co-infections have consistently been reported to be higher than those observed for other respiratory infections and it is not easy to determine whether such co-infections are sequential infections or simultaneous viral infections[37].

High frequencies of co-infections by other respiratory viruses in HBoV+ cases were confirmed in this study with a rate of 75% (15 of 20). This was similar to the 75% reported by Allander et al., in Sweden[4], and was comparable with those reported in the literature, with rates of co-infections ranging from 35% to 90%[6,10,17,25,34]. Noticeably about 66.7% of the co-infections in our study are HBoV-RSV co-infections (Table 2). Others also recognized elevated percentages of co-infection with RSV among HBoV+ patients[10-14]. This high rate of co-infection could be an effect of co-circulation, although an interaction between these viruses cannot be disregarded. In contrast, Lin et al.[38] and Zhao et al.[39] did not observe any co-infections in positive HBoV samples in their study.

In our study, the main clinical manifestations in HBoV+ patients included
cough (100%) and wheezing (90%). Our data were consistent with many other studies that support the fact that wheezing was one of the most common manifestation presented in HBoV+ patients\[6,29,34,35,40\].

In agreement with previous studies\[25,40-42\], there is no significant differences were found in term of frequencies of respiratory manifestations between patients with single HBoV infection and patients with co-infection with other viruses (Table 3). Clearly, our results indicated potential co-infections will not influence clinical outcome of HBoV infection in respiratory tract. This finding strongly suggested the pathogenic potential of HBoV in young children with ARTIs. Many authors reported that patients with HBoV without concurrent infections by other respiratory viruses, tended to shed higher HBoV loads\[6,43\], a feature strongly suggestive of a causal role for this agent in acute symptomatic infections. These findings are in agreement with our results that revealed HBoV viral loads to be significantly higher in patients with single HBoV infection than patients with co-infection with other viruses (p=0.006) (Table3). These findings are also consistent with a Norwegian study that reported detection of HBoV alone and a high viral load were associated with respiratory tract infection\[41\].

We also investigated the relationship between HBoV viral load and frequencies of respiratory manifestations and we found that high HBoV viral load led to more severe lower respiratory tract manifestations (Table 4). This result was consistent with the result by Deng et al.\[46\]. However, other groups have been unable to establish a link between HBoV viral load and disease severity as well\[11,12,38,44\].

Although there is no doubt that HBoV is a common human virus acquired early during life, and the seroepidemiology and prevalence studies have shown evidence for widespread exposure to the virus, the causative role of HBoV in respiratory tract disease is still debatable and under investigation\[45\]. The possible reasons for this debate are: frequent detection of this virus in co-infection with other respiratory viruses of well-established pathogenic role\[6,16,17,25,34\], frequent detection in asymptomatic children's respiratory tract secretions\[46\] and persistent virus shedding in respiratory secretions after resolution of disease\[47\]. In addition, HBoV is fastidious to propagate in conventional cell cultures and an experimental infection model is lacking, what limits advances in understanding and makes it difficult to draw conclusions about its pathogenicity\[21\]. While there has been one recent successful report of propagation of HBoV in cell culture, this technique has proven difficult to replicate\[8\]. Therefore, additional evidence and studies are needed throughout the world to gain a better understanding of this virus and to determine whether HBoV played a causative role in these co-infections or acted as an exacerbation factor. Also, more studies are required focusing on various aspects of this infection, including serology, in vitro culture and animal models.

**CONCLUSION**

This is the first study of HBoV in Benha. Our study conclude that HBoV is one of the most prevalent respiratory viruses in children and our findings are consistent with the potential etiologic role for HBoV in respiratory tract disease in young children based on the following data:

1- No significant differences were found in term of frequencies of respiratory manifestations in patients with single HBoV infection and patients with co-infection with other viruses.

2- The median viral load in patients with sole HBoV infection was significantly higher than those with co-infection with other respiratory viruses.

3- Frequency and severity of lower respiratory manifestations correlate with HBoV high viral load.

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فيروس بوكا البشري بين الأسباب الفيروسية للالتهابات الجهاز التنفسي للأطفال

في مستشفى الناجح الجامعي

فاطمة عبد المجيد طلاب، طبيبة أمراض، عنت حسين عمر

الخلفية / الهدف: فيروس بوكا البشري هو أحد الفيروسات التي تم اكتشافها حديثًا والذي يرتبط وجوده بالالتهابات الحادة في الجهاز التنفسي للأطفال، وكأن الهدف من هذه الدراسات هو معرفة الخصائص الواعية والأكليتية لهذا الفيروس وعلاقتها بكلية الفيروس في إفرادات الجهاز التنفسي عند الأطفال المصابة بالالتهابات الحادة في الجهاز التنفسي في مستشفى الناجح الجامعي.

الطريقة: عدا عدد هذه الدراسة في قسم علم الأحياء الدقيقة والمناعة، كلية الطب، جامعة الناجح، في الفترة من سبتمبر 2010 إلى يوليو 2011 على 400 عينة من الخلايا الأحادية الفيروسكوبية من الأطفال المصابين بالالتهابات الحادة في الجهاز التنفسي، الذين يتردون على العيادات الخارجية للأطفال، وقسم طب الأطفال، في مستشفى الناجح الجامعي، وقد قاموا بإعدادهم من 1 شهر إلى 12 سنة. وقد تم إختبار جميع العينات بواسطة اختبار الديلا彩色 للكشف عن فيروس بوكا البشري، وأيضاً اختبار النتائج الناعم المباشر لكشف عن أهم الخصائص الفيروسية للالتهابات الجهاز التنفسي في الأطفال، وهي فيروسات الطبل والتحضير والفيروسات الحادة، والأنفلونزا ألف وباء، والبارا إنفلونزا 2,3,4,5,6 من الخلايا الخلايا، وأيضاً اختبار الفيروسات المشتركة.

النتائج: نتج عن هذه الدراسة أن فيروس بوكا البشري هو الأكثر انتشاراً حيث الكشف عن الجينوم الخاص به في 10% من العينات، وقد كانت 75% من الحالات الإيجابية لفيروس بوكا البشري في عديد مشتركة مع واحد من العناصر الفيروسية أعلاه. في حين كان اللفيروس الوحيد في 25% منها. وكانت معظم العدوى (19,7%) مشتركة مع فيروس الخلايا متعددة الألوية، وكان متوسط عمر الحالات الإيجابية 19.1 شهر حيث كانت الحالات السوائل من عمر عائلي. وقد تم اكتشاف أغلب الحالات في فصول الشتاء. لم تسفر هذه الدراسة عن أي علاقة ذات دالة إحصائية بين مجموعات العدوى المشتركة و العدوى المتفردة من فيروس بوكا البشري من حيث العمر أو الجنس أو الأعراض المرضية. وقد وجد أن متوسط كمية الفيروس في إفرادات الجهاز التنفسي في مجموعة العدوى المتفردة أكبر منه في مجموعة العدوى المشتركة كما أثبتت الدراسة وجود علاقة ذات دالة إحصائية بين كمية الفيروس وظهور وشدأ أعراض الجهاز التنفسي السفلي.

الخلاصة: فيروس بوكا البشري هو أحد الفيروسات الأكثر انتشاراً بين الفيروسات التي تسبب الجهاز التنفسي، ولعب دورًا هاماً في حدوث التهابات الحادة في الجهاز التنفسي في الأطفال في مستشفى الناجح الجامعي.