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Detection of Human Bocavirus in Nephrotic Syndrome Children with Acute Upper Respiratory Tract Infections

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Abstract

Background Nephrotic syndrome (NS) is a frequent chronic illness marked by changes in permselectivity at the glomerular capillary wall, as a result, it is unable to limit protein loss through the urine. Bacterial and viral infections are more common in patients with NS. Human Bocavirus (HBoV) is an emerging pathogen suspected to cause respiratory and GIT infections in children.

Objective To investigate the frequency of HBoV in children with NS who have acute upper respiratory tract infections, and compare it with normal controls.

Methods A case-control study carried out on 120 nasal swabs from children divided into three groups; 40 children each group (nephrotic syndrome and immunocompetent children with acute upper respiratory tract infections, and apparently children in good health without respiratory infections as control group). Viral DNA extracted from these samples and HBoV detected using real-time polymerase chain reaction.

Results HBoV was detected in 30 (75%) of patients with NS, and 18 (45%) in normal children with acute upper respiratory infections, while it was 3 (7.5%) in apparently healthy control group. The mean cycle threshold (CT) of HBoV in the three groups were 18.99 in nephrotic patients, 20.21 in normal children with acute upper respiratory infections and 24.33 in control group

Conclusion HBoV is relatively common among nephrotic children with acute upper respiratory infection, with a lowest CT (high viral load) as compared to the apparently normal children with acute upper respiratory infections and children in good health.

Keywords Nephrotic syndrome, Human Bocavirus, acute upper respiratory infections, children

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List of abbreviations: CCL = Chemokine (C-C motif) ligand, CT = Cycle threshold, HBov = Human bocavirus, IC = Internal control, IFN = interferon, IL = Interleukin, NS = Nephrotic syndrome, PCR = Polymerase chain reaction, SD = Standard deviation, TGF = Transforming growth factor

Introduction

Nephrotic syndrome (NS) is a prevalent chronic illness characterized by permselectivity changes at the glomerular capillary wall, as a result, it is unable to limit protein loss through urine. Nephrotic range proteinuria is defined as proteinuria exceeding 1000 mg/m² per day or spot (random) urinary protein-to creatinine ratio exceeding 2 mg/mg. The proteinuria in childhood NS is relatively discerning, mostly composed of albumin [(1)]. Patients with NS, bacterial and viral infections are more common in them. Urinary losses of immunoglobulins, complement, and properdin cause an increase

in infection susceptibility. Altered T-cell mechanisms, the usage of immunosuppressive therapy for a long time, and the presence of edema also contribute to infections [2].

Because of the immunodeficiency status in patients with NS, respiratory infections are the most common complications among them [3].

Human Bocavirus (HBoV) was first described by Allander and colleagues in 2005 [4]. The HBoV virions are icosahedral, non-enveloped and small, roughly 18-26 nm in diameter and their linear single-stranded DNA genome is 5543 bp in length [5]. HBoV has been linked to the upper and lower respiratory tract in a number of studies. Cough, fever, rhinorrhea, asthma exacerbation, bronchiolitis, severe wheezing, and pneumonia are the most commonly described clinical manifestations of HBoV infection in this regard [4,6-8].

The objective of this study was to investigate the frequency of HBoV in children with NS who have acute upper respiratory tract infections, and compare it with normal controls.

Methods

A case-control study carried out on 40 children to each (NS children with acute upper respiratory infection, normal children with acute upper respiratory apparently healthy children without respiratory infections as control group) aged 3-18 years admitted to Al-Imamein Al-Kadhimein Medical City, Central Teaching Hospital of Pediatric, Children Welfare Teaching Hospital at (Baghdad Medical City), and Al-Karama Teaching Hospital – Baghdad. Nasal swabs were collected during winter and autumn of 2020. This study was approved by the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University (approval no. 327 in 24/12/2019). Nasal swabs were placed in 1 ml virus transport media (VTM) tube (Heinz Herenz, Germany), which provided with the swab and stored at (-20C°) and the DNA extraction was done by using Viral Gene-spin™ Viral DNA/RNA Extraction Kit (Intron, Cat No 17151, Korea). Each sample was analyzed for the presence of HBoV by using ARVI-screen-FRT polymerase chain reaction (PCR) kit (R-V57-100-F) (AmpliSens, Russia). This kit is an in vitro nucleic acid amplification test for multiplex detection and identification of specific nucleic acid fragments of pathogens that cause acute respiratory viral infections. In this study, only the reagents of HBoV were used. The PCR mix (total volume 25 µl) composed of 10 µl of PCR-mix-1-FL-F, 5 µl of PCR-mix-2-FRT, Polymerase (TaqF) (0.5) µl, 3 µl were added of internal control, from this mixture, 15 µl is taken and 10 µl of the sample or positive control was added to it. PCR procedures were carried out on a real-time PCR detection system (Sa-cycler-96, Italy). Analysis of the PCR data was performed with computer software provided by the instruments company (Sa-cycler-96, Italy). Cycling conditions for the real time-PCR procedures were 1 cycle (95°C, 15 min) followed by 10 cycles (95°C, 10 sec) (54°C, 25 sec) (72°C, 72 sec) and then 35 cycles (95°C, 10 sec) (54°C, 25 sec) (72°C, 72 sec). The results were based on the number of positive cases for HBoV in each group and make a comparison between the mean of cycle threshold (CT) values in the three groups, and find out which group has the lowest CT value (higher viral load).

Statistical analysis

The data were processed using statistical package for social sciences (SPSS) version 16.0.0, and Microsoft Excel 2010. The data of the current study were scrutinized carefully in terms of being parametric or non-parametric using normality tests. Analysis of variance (ANOVA) test was used to measure the difference in means between groups.

Results

The age of NS ranged from 3-16 years; their mean age was 9.15 years. The respiratory presentations, sex and age of NS patients whom positive for HBoV were summarized in table (1).

The positive cases with HBoV were 30 (75%) out of 40 in nephrotic patients with acute upper respiratory infection, 18 (45%) in normal children with acute upper respiratory infection

and 3 (7.5%) in apparently healthy control group. The mean of CT values of HBoV in three groups were 18.99 in nephrotic patients, 20.21 in normal children and 24.33 in control group as shown in table (2).

Table 1. Age, sex and respiratory presentations of nephrotic syndrome patients whom +ve for HBoV

Parameter		NS, URTI +ve HBo N (%)	Normal, URTI +ve HBov N (%)	Normal +ve HBov N (%)
Sex	Male	15 (50.0)	12 (66.66)	2 (66.66)
	Female	15 (50.0)	6 (33.33)	1 (33.33)
p. value		1.030	0.222	0.977
Age (year)	3-5	4 (13.33)	7 (38.88)	2 (66.66)
	5-10	16 (53.33)	6 (33.33)	1 (33.33)
	10-20	10 (33.33)	5 (27.77)	0 (0)
p. value		0.07	0.221	0.977
Respiratory presentations	Common cold	25 (83.33)	10 (55.55)	---
	Pharyngitis	10 (33.33)	8 (44.44)	---
p. value		0.004	0.746	---

NS: Nephrotic syndrome, URTI: Upper respiratory tract infection

Table 2. The mean of cycle threshold values of HBoV in the three groups

Group	HBov +ve N (%)	Cycle threshold mean±SD	P value
Nephrotic syndrome with URTI	30 (75.0)	18.99±2.27	
Normal with URTI	18 (45.0)	20.21±1.75	0.222
Normal	3 (7.5)	24.33±1.31	

URTIs: Upper respiratory tract infections, SD = standard deviation

Discussion

There were several Iraqi studies, the results of which showed the presence of HBoV among children in Iraqi society [9-11]. In this study, 3/4 of NS patients have HBoV infection, also HBoV CT was lowest among patients with NS. There were more than one study linking between viral respiratory infection and exacerbation of the condition of patients with NS [12-14]. As for the direct relationship between HBoV and NS, to the best of our understanding, there is no study so far that explains the details of the mechanism of infection with this virus in patients with NS, but there is a study that showed the relationship between one of the important respiratory viruses, which is the respiratory syncytial virus (RSV) and NS. Rats infected with RSV developed proteinuria, according to the study, this was followed by a significant amount of podocyte destruction, and very modest alterations in renal tubular epithelia cells and mesangial cells. The connection between rat immune responses to RSV and nephrotic infection was explored in this study. Following RSV infection, blood levels of the cytokines (interleukins) IL-6, IL-17, and transforming growth factor (TGF)- were raised, and serum levels of IL-6 and IL-17 were higher following RSV re-infection than following RSV main infection. These findings suggested that abnormal adaptive immune responses to viral infection may exacerbate nephrotic damage.

RSV infection causes the production of cytokines such as type I (interferon) IFN and IFN-, IL-6, IL-8, IL-10, IL-13, and IL-17, as well as chemokines such as Chemokine (C-C motif) ligand (CCL3, CCL2, and CCL5) [15]. According to Turner et al. (2010), cytokines such as IL-17, IL-6, and IL-21 can bind to receptors on mesangial cells and renal tubular epithelial cells, causing chemokine production and neutrophil and monocyte recruitment to the kidney. These processes have the potential to cause pathological injury of kidney [16]. Respiratory tract infection a risk factor for the onset and relapse in NS patients [17-19]. Perhaps the HBoV performs the same mechanism when infecting patients with NS, as it is one of the respiratory viruses or there was a co-infection with other viruses.

There was a noticeable difference in the number of positive cases in the three groups, which the highest number and lowest CT (high viral load) in nephrotic group children and the expected reasons for this in the presence of a weakened immune system, exposure to HBoV during immunosuppression can lead to persistent infection and prolonged viral shedding [20].

In conclusion, HBoV had a wide distribution and lowest CT (high viral load) among nephrotic children with acute upper respiratory infection in compared to the normal children with acute upper respiratory infection and healthy children.

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Author contribution

The authors, altogether, conceived and planned the study. The experiment was done by Dr. Lazim under supervision of Dr. Ali and Dr. Salim.

Conflict of interest

There is no conflict of interest.

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