Detection of Human Bocavirus in Children Suffering from Respiratory Tract Infection in Diyala Province

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Abstract

Background: Human Boca virus (HBoV) is one of the viral infections present worldwide.
Objective: To determine the role of HBoV in respiratory tract infection.
Patients and Methods: Cross sectional study consists of 160 (Pharyngeal secretions and blood) samples were collected from children who attended Al-Zahraa Hospital for respiratory complaints during the period from October 1st 2018 to April 30th 2019, including both genders and ages ranging from (3 months to 10 years). The samples were divided into two groups. First group consisting of (80) blood samples for patients, while the second group (80) sample swabs from the pharynx.
Results: The prevalence of HBoV using PCR technique was 38.3%. Those less than 2 years showed the highest rate of infection 43.5%, followed by 5-7 years 42.9% and 2-4 years 33.3% while the lowest percentage in the age group (8-10 years) 28.5%. A high rate of IgG was recorded in age group less than 2 years old 51.3%, followed by the category of 5-7 and 2-4 years with 28.6% and 22.2% respectively.
Conclusion: A high rate of HBoV infection in children with respiratory illness in Diyala province**.**

Keywords: Human Bocavirus, respiratory tract infections, PCR, ELISA.
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Introduction

Pathogenic viruses are a prominent causes of lower respiratory infection, [1,2].Human bocavirus (HBoV) is one of the viral infections present worldwide. It was first identified in 2005 by examining the nasal pharyngeal secretions of children with respiratory infections. It belongs to the family Parvoviridae and subfamily Parvovirinae . Human bocaviruses are single strand DNA viruses. Four serotypes of HBoVs have been described [2,3] Three species HBoV2, HBoV3, and HBoV4 were

found mainly in stool samples which cause gastroenteritis [3-7]. While Human bocavirus 1 causes upper and lower respiratory tract infection(RTI). Infection with this type of virus is common all over the world and infection occurs throughout the year but prevalent during winter and spring. Epidemiological revisions indicated that the HBoV1 virus is worldwide spread and other serotypes 3-4 viruses are also common in children with respiratory system infections [5-10]. The prevalence HBoV1 DNA in young children with RTI is about 10% but in some researches showed up to 33% [1,11.12]. The most common clinical diagnoses associated with HBoV1 RTI are upper respiratory tract infections, bronchiolitis, pneumonia, bronchitis, asthma exacerbation and pharyngitis [7-13] . Human bocavirus 1 is identified by several methods, including serological methods like western blotting, immunofluorescence assays [8,14-17], as well as enzyme immunoassay (EIA) and enzyme linked immunosorbent assay to detect IgG and IgM antibodies [9,18-23]. Atyah in 2017 in Iraq showed HBoV infection was 48/195 24.62% using Real Time PCR technique [12].Molecular methods used to detect HBoV1 virus using Polymerase Chain Reaction (PCR) technique are the most accurate and modern [9,15,24] .The objective of this study is to define the role of HBoV in respiratory tract infection using PCR methods and ELISA.

Patients and Methods

Sample size and design: study was done in Al Zahraa hospital / Diyala province for the period from October 1st 2018 to April 30th 2019. One -hundred sixty samples collected by the authors from children . These samples were divided into 80 blood samples and 80 pharyngeal swabs. The blood samples consist of 35 females and 45 males. The swabs samples consist of 35females and 45 males,. Samples were collected from hospitalized children, outpatient clinic attendees suffering from respiratory infections who were aged from 3 months to 10 years. A three ml of venous blood withdrawn by using plastic medical syringes. Blood has been expended in test tubes and left for 30 minutes at room temperature to coagulate. The serum was separated by a centrifuge for 5 minutes (3000 cycles / min) and stored at - 20°C until use for HBoV IgG and IgM antibodies finding. The swabs were collected by taking a swab from the pharynx and a normal saline solution was added for sterilization and stored at -70°C until usage for PCR.

Serological Procedure: Human Bocavirus IgG and IgM antibodies levels were detected by double antigen sandwich enzyme –linked immunosorbent assay . The ELISA Kits provided by GenAsia Biotech (Cat.no. GA-E5461HM) in all patient's serum samples agreeing to the manufacturer's directions.

Qualitative polymerase chain reaction: Viral nucleic acid was extracted from 100 µl of the samples using RIBO – PreP nucleic acid withdrawal Kit Cat. No. GA-E5460HM Amplisens biotechnologies ,Russia agreeing to the manufacturer's guidelines. The existence of HBoV genomes were identified from the removed nucleic

acid by qualitative polymerase chain reaction (PCR) with exact primers the NP-1 primers BoV18 and BoV542R [6,9] Table(3). Polymerase chain reaction combination was organized with 20 µl of lyophilized Master Mix (Korea /Bioneer), 13µl of nuclease unrestricted water, 1µl of each primer at 10 pmol/µl, and 5µl of DNA template, $MgCl_2$, KCl ,dNNTPs, Tris-HCl PH 9.0 ,Tag DNAPolymerase, Stabilizer tracing dye PCR Thermocycling circumstances of 95°C (initial denaturation) for 10 min, monitored by 50 cycles of denaturation at 94°C for 30 sec. and toughening at 53 C° for 40 sec, then the final delay step at 72°C for 1 min settled the reaction program.

Ethical consent: The study protocol and the questionnaires were directed agreeing to the principles of the Declaration of Helsinki, as well as revised and agreed by the Ethical Research Committee of College of medicine, University of Diyala. Verbal consents were taken from the parents and caregivers of children enrolled in the study.

Statistical analysis

The data were processed and analyzed using the Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA. The results were expressed using percentage and frequencies. A p-value <0.05 was considered significant.

Results

Prevalence of HBoV- IgG for study samples: The results of our work elucidated seropositivity IgG antibody test for infected patients with HBoV for 28 samples comprised 35%.

Present study group for IgG HBoV antibodies according to age groups: The results of the present study showed that the age group less than 2 years documented the highest percentage for IgG 51.3%, while the lowermost percentage was in the age group 2-4 years 22.2%. The age group 5-7 years recorded the percentage 28.6 %. No IgG was reported for 8-10 year's age group, with an important variance when matched with control group (P<0.05) Table(1).

Table (1) : The positive & negative carrier for IgG HBoV antibodies according to age groups

Groups	Positive		Negative		Total	
	No	%	No	%	No	%
Less than 2 years	20	51.3	19	48.7	39	48.8
2-4 Year	6	22.2	21	77.8	27	33.8
5-7 Year	2	28.6	5	71.4	7	8.8
8-10 Year	0	0.0	7	100	7	8.8
Total	28	35.0	52	65.0	80	100

* P Value =0.016

The present study group for HBoV IgG antibodies according genders.The results of this study showed insignificant variance in HBoV infection in both genders. The proportion of infection was 37.8% for boys and 31.4% for girls as in Table(2).

Table(2): The positive and negative carrier for IgG HBoV antibodies for both genders

	Positive		Negative		Total		P Value
Genders	No	%	No	%	No	%	
Female	11	31.4	24	68.6	35	43.8	
Male	17	37.8	28	62.2	45	56.3	P = 0.55
Total	28	35.0	52	65.0	80	100	

The occurrence of HBoV- IgM antibodies for study samples The results of the present study revealed seropositivity IgM antibody test for infected patients with HBoV at 7 samples and 8.8% .

Table(3): Shows the primers used for HBoV DNA amplification

Primers	Sequence(5'-3')	Size (bp)
HBoV188F	GAGCTCTGTAAGTACTATTAC	354
HBoV542R	CTCTGTGTTGACTGAATACAG	354

Molecular Diagnosis of HBoV for Infected Children: This study disclosed that among 80 samples gained from children with (RTI), there were 31 positive results for HBoV DNA (38.8%) and 49 (61.2%) samples were negative by qualitative PCR. This designates high infection rate.

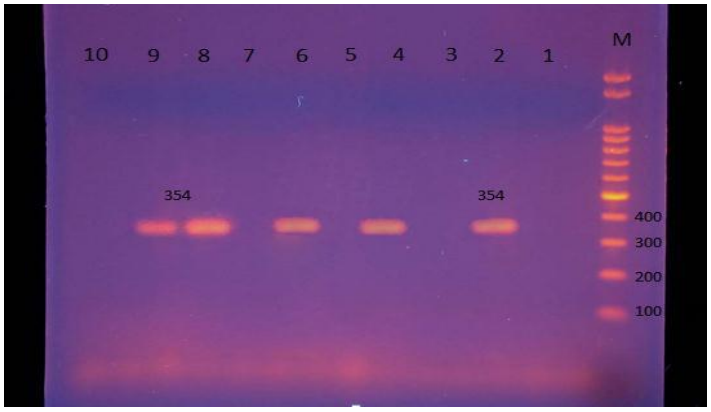

Figure (1): Gel electrophoresis for PCR produce to detect Human bocavirus, 354 bp, via (1.5%) agarose for 45 minutes at 75 volt. M – 100 bp ladder. (2, 4 ,6,8 ,9) .PCR produce of positive isolates from samples, negative (1,3, 5,7 ,10)

Demographic characteristics of children with (RTI) and their association to HBoV: Variation of HBoV infection between different age groups.The results of the current study demonstrated that the age group less than two years who were 39 cases, 17 of them were positive with percentage 43.5%. While age group 2-4 years who were 27 samples, 9 of them were positive 33.3%. While the age group 5-7 years was 7 samples, 3 were positive 42.9%. Lastly, the age group 8-10 years 7, two of them were positive 28.5%, with insignificant variance (P>0.05) as shown in Table (4).

Table (4): Positive and negative cases according to age groups

	Positive		Negative		Total		P Value
Age Groups	**No**	**%**	**No**	**%**	**No**	**%**	
Less than two years	17	43.5	23	59.0	39	48.8	
2-4 Year	9	33.3	19	70.4	27	33.8	P=0.55
5-7 Year	3	42.9	4	57.1	7	8.8	
8-10 Year	2	28.5	3	42.9	7	8.8	
Total	31	38.8	49	61.3	80	100	

Relationship between HBoV infection and gender: The outcomes of the current study showed insignificant variances in HBoV infection in both genders. The proportion of infection among boys was (40.0%) matched with (37.1%) for female as shown in Table (5). Our study showed that the incidence of infection between males and females is Congregated.

Table (5): The positive and negative samples for genders

	Positive		Negative		Total		P Value
Genders	**No**	**%**	**No**	**%**	**No**	**%**	
Female	13	37.1	22	62.9	35	43.8	P= 0. 79
Male	18	40.0	27	60.0	45	56.3	
Total	31	38.8	49	61.3	80	100	

Discussion

The occurrence of HBoV differs among different nations of the world due to climatic and topographical influences as well as health care or cultural and socioeconomic level. Numerous studies have designated that HBoV occurrence is higher in winter [10,11].The results of this study displayed that the proportion of infection was 38.8% by qualitative PCR. In earlier study done by Atyah in 2017 in Iraq showed HBoV infection was 48/195 24.62% using Real Time PCR technique [12]. A study done in Egypt to identify the DNA of the virus in nasopharyngeal swab in 2016 revealed that the infection proportion was 56.8% for 95 samples [13]. A study achieved in China in 2012 using Real Time PCR showed the occurrence rate was 24.6% [14].This variance can be elucidated by several factors including sampling procedures, study groups, climate disparities and the sensitivity of several tests to identify the virus [15]. The outcomes of our study revealed that the age group less than two years verified the highest rate 43.5% in PCR results, followed by 42.9% in age group 5-7 years, and 33.3 % in age group 2-4 years). While the lowermost

proportion was 28.5% in age group (8-10) years. A study achieved by Atyah in 2017 in Iraq showed that the frequency of the virus in the age group 1-2 years was 68.75% followed by the age group 2-5 years 29.17% and the age group 5-15 years 2.08% [12] .Another study observed a high incidence of HBoV in children aged less than1 year) 57.9%[16]. Another study showed the rate of infection in the age group less than two years 63.91% [17].Primary infection with HBoV occurs early in children age less than 2 years) [18] .Newborns are less likely to have the infection because of protective antibodies obtained from mother [19].The results of the this study relative to gender showed that the proportion of infection in males 40.0%, while the rate of infection in females 37.1% and infection between males and females was congregated. Our results differ from study performed in Egypt where the infection in males consisted 78.86%, and in the females 21.14% [16]. A study performed in China in 2016 appeared infections in males 65. 9% while in female was 34.1% [17] which showed no statistical differences. Concerning IgG seropositivity result was 35% with an important variance when matched with control group. Our results agreed to that performed in China by Lin et al which was 31% of children with lower respiratory tract infections. All age groups of patients showed an upper level of antibodies to HBoV IgG using the ELISA technique and using human bocavirus VP2 virus-like elements [20]. Our study is inconsistent with that conducted in Finland where IgG antibody detected in 111/258 43% [21]. The results of the present study revealed that the age group less than two years recorded the highest rate for IgG 51.3%, while the lowest rate was in the age group 2-4 years 22.2%. The age group 5-7 years documented 28.6%. No IgG was reported in the 8-10 year's age group. Sero-epidemiological study in Jamaica in 2012 specified that more than 80% of children are liable to HBoV at the age of two years [22].Another study in Italy in 2012 specified that the occurrence of IgG was 73.7% in children aged one day to 5 months and 51.4% in the 6-11 month age group, 64.2% in the 2-4 years and the high proportion of immunoglobulin IgG in the age group 5-9 years 96.4% [23]. Our results showed the seropositive for IgG antibody amongst males 37.8 % while the rate of IgG in females 31.4%. This is in agreement with a study performed in Jamaica [22]. The results of this study revealed seropositivity IgM antibody for infected children with HBoV 8.8% , these results were slightly less than that reported in Egypt in 2017 where, the occurrence of IgM 16 / 123 13% by ELISA [16]. As well as in Finland and Germany [24,25]. This differences may be due to social and environmental conditions, as well as the type of kit used for diagnosis and sample preparation from the infected patients.

Conclusions

The study showed a high rate of HBoV infection in children with respiratory infections in Diyala province, the age groups less than 2 years had the highest incidence of

infection and the lowest incidence was in the 8-10 years age group using the PCR method.

Recommendations

The clinician should be aware about human bocavirus in children with respiratory tract infection at least in our locality.

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