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DETECTION OF HUMAN BOCAVIRUS IN IRAQI PEDINTRIC PATIENTS SUFFERING FROM RESPIRATORY INFECTION USEING ELISA AND PCR METHODS

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ABSTRACT

This study was conducted in Al Muqdadiya district in Diyala province. A total 180 samples were collected from pediatric patients who were in Al-Zahra Hospital for gynecology and pediatrics.Were Included both sexes and ages ranging from (3 months to 10 years) whose suffering from respiratory infections. The study samples were divided into two groups. First group consisting of (80) blood samples for patients and (10) samples as control group for healthy people, the second group (80) Sample swabs from the pharynx and control group composed of (10) healthy people. The results showed the prevalence of (HBoV) using PCR technique was (38.3%) with significant difference (P<0.05) when compared with control group, age group (3 months -1years) recorded the highest rate of infection (43.5%), followed by (5-7) years (42.9%), and from (2-4) years (33.3%) while the lowest percentage in the age group (8-10 years) (28.5%) with non a significant difference between age groups (P>0.05).The percentage of infection among males was (40.0%) and females (37.1%) with no significant difference (P>0.05). The study demonstrated an increase the antibodies to HBoV in patients compared to healthy, The IgG ratio was (35.0%) with a significant difference when compared with control group (P <0.05). A high rate of IgG was recorded in age group (3months -1 years) (51.3%), followed by the category of (5-7) years consisted (28.6%) and (2-4) years consisted (22.2%), while in age group (8-10) years was not detected, with a significant difference

between age groups (P<0.05). The results illustrated an increased in the percentage of infection among males was (37.8%) while in females was (31.4%) with no significant difference (P>0.05).The prevalence of IgM antibody was (8.8%) and a significant difference when compared with control group(P<0.05).

KEYWORDS: Human Bocavirus (HBoV), respiratory tract infections, PCR, Elisa.

INTRODUCTION

Infectious microbes are the most important causes of respiratory infections It is largely responsible for deaths, especially in newborns and young children The microorganisms that cause inflammation are pathogenic bacteria, mycoplasma, fungus.

Pathogenic viruses are a prominent causes of lower respiratory infection, include Human Parainfluenza viruses (HPIV), Respiratory syncytial viruses (RSV), Human adenoviruses (HAdV), Human Coronaviruses (HCoV), rhinoviruses, Human MetaPneumo viruses (HMPV), Human bocaviruses (HBoV), influenza viruses.[1]

Human bocavirus (HBoV) is one of the most common viral infections worldwide. It was first identified in 2005 by examining the nasal pharyngeal secretions of children with respiratory infections. HBoVs belong to the family Parvoviridae and subfamily Parvovirinae. HBoVs are single strand DNA viruses. Recently four types of HBoVs have been described. In 2005 HBoV1 was discovered by Alander and his group in children with respiratory tract infections (RTI).[2] The other three species HBoV2, HBoV3, and HBoV4 were found mainly in stool samples.[3] HBoV1 causes upper and lower respiratory system infection. The other three species detected in stool samples cause gastroenteritis.[4]

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 studies indicated that the HBoV1 virus is globally spread and that three to four viruses are common in children with respiratory system infections.[5,6] The prevalence HBoV1 DNA in young children with RTI is about 10% but in some research showed up to 33%.[1] The most common clinical symptoms associated with HBoV1 respiratory tract infections are upper respiratory tract infections, bronchiolitis, pneumonia, bronchitis, asthma exacerbation, rhinitis, Pharyngitis.[7] HBoV1 is diagnosed by several techniques, including serological methods including western blotting, immunofluorescence assays[8], as well as enzyme immunoassay (EIA) and enzyme

linked immunosorbent assay (ELISA) to detect IgG and IgM antibodies.[9] Molecular methods used to detect HBoV1 virus using Polymerase Chain Reaction (PCR) technique are the most accurate and modern.

The aim of this study is designed to determine the role of HBoV in respiratory tract infection using PCR methods and to study some of the immune aspects associated with this virus including IgG and IgM antibody in serum using (ELISA) method.

MATERALS AND METHODS

Collection of sample: Our study was conducted in Al Muqdadiya district in Diyala province for the period from November 2017 to May 2018. This study included (180) samples of females and males patients. The samples were divided into (90) blood samples and (90) Pharyngeal swabs. The blood samples consist of (35) females and (45) males. The study also included a control group for healthy people consisting of (10) samples (5) females and (5) males. The swabs samples consist of (35) females and (45) males, and the control group consisted of (10) samples (5) females and (5) males. Samples were collected from hospitalized patients outpatient clinic visitor suffering from respiratory infections who were aged from 3 months to 10 years.

Blood samples were collected by drawing 3 ml venous blood 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 detection. 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 use for PCR**.**

Serological Technique

HBoV IgG and IgM antibodies levels were determined by double antigen sandwich enzyme – linked immunosorbent assay (ELISA) method. the ELISA Kits supplied by GenAsia Biotech (China) in all patients serum samples according to the manufacturer's instructions.

Qualitative polymerase chain reaction (PCR)

Viral nucleic acid was extracted from 100 µl of the samples using RIBO –PreP nucleic acid extraction Kit (Amplisens biotechnologies ,Russia) according to the manufacturer's instructions. The presence of HBoV genomes were detected from the extracted nucleic acid

by qualitative polymerase chain reaction (PCR) with specific primers the NP-1 primers BoV18 and BoV542R[2] (table1). PCR mixture was prepared with 20 µl of lyophilized Master Mix (Korea /Bioneer), 13µl of nuclease free water, 1µl of each primer at 10 pmol/µl, and 5µl of DNA template. Thermocycling conditions of 95°C (initial denaturation) For 10 min, followed by 50 cycles of denaturation at 94°C for 30 sec, and annealing at 53 C° for 40 sec, then the final extension step at 72°C for for 1 min concluded the reaction program.

RESULTS

Table 1. Primers used for HBoV DNA amplification

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

Molecular Diagnosis of HBoV Virus for Infected Children: This study showed that among 80 samples obtained from children with (RTI) there were 31 positive results for HBoV DNA (38.8%) and 49 (61.3%) specimens were negative by qualitative PCR. While all control group were negative for HBoV. With a significant difference when compared with control group P<0.05 (Table 2). This indicates to high infection rate.

Table. (2): Shows the number of positive and negative cases by PCR technique.

Groups	Positive		Negative		Total		P. Value
	No	%	No	%	No	%	
Patients	31	38.8	49	61.3	80	100	P=0.015
Control	0	0.0	10	100	10	100	
Total	31	34.4	59	65.6	90	100	

N: number

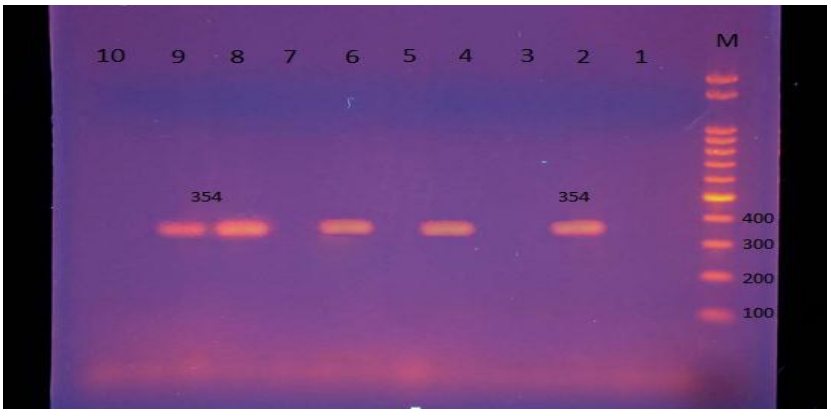

Figure 1: Gel electrophoresis for PCR product to detection Human bocavirus, 354 bp, using (1.5%) agarose for45 minutes at 75 volt. M – 100 bp ladder. (2,4 ,6,8 ,9) PCR product of positive isolates from patients, negative (1,3, 5,7 ,10).

Demographic characteristics of patients with (RTI) and their relationship to HBoV.

Variation of HBoV infection between different age groups

The results of the current study demonstrated that the age group (3 months -1 years) who were (39) cases, 17 of them were positive with percentage (43.5%) . And for age group (2-4) years who were (27) cases, 9 of them were positive (33.3%). While the age group (5-7 years) was (7) cases, 3 were positive (42.9%). Finally the age group (8-10) years (7) 2 of them were positive (28.5%). with no significant difference (P>0.05) as shown in table (3).

Table. (3): positive and negative cases according to age groups.

Age Groups	Positive		Negative		Total		P Value
	No	%	No	%	No	%	
3 Month -1 Year	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 results of the current study showed no significant differences in HBoV infection in both sexes The percentage of infection among males was (40.0%) compared with (37.1%) for Female as shown in Table (4). This study showed that the incidence of infection between males and females is Converged.

Table. (4): Showed positive and negative patients for gender.

Gender	Positive		Negative		Total		P Value
	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	

Elisa Results

Prevalence of HBoV- IgG HBoV for study samples: The results of the our study clarified seropositivity IgG antibody test for infected children with HBoV for 28 cases consisted (35%) with a significant difference when compared with control group (P<0.05) as in table (5).

Table. (5): Positive and negative carrier of IgG HBoV antibodies.

Groups	Positive		Negative		Total		P Value
	No	%o	N	%	No	%	
Patients	28	35.0	52	65.0	80	100	P=0.024
Control	0	0.0	10	100	10	10	
Total	28	31.1	62	68.9	90	100	

Current study group for IgG HBoV antibodies according to age groups: The results of the current study showed that the age group (3 months -1 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 recorded the percentage (28.6%). No IgG was reported for (8-10) year's age group. With a significant difference when compared with control group (P<0.05) (Table 6).

Table. (6): Positive and negative carrier for IgG HBoV antibodies according to age. Groups.

Age Groups	Positive		Negative		Total		P Value
	No	%	No	%	No	%	
3 Month -1 Year	20	51.3	19	48.7	39	48.8	P=0.016
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	

Current study group for HBoV IgG antibodies according genders

The results of the study was showed no significant difference in HBoV infection in both sexes. The percentage of infection was 37.8% for males and 31.4% for females as in table (7).

Table. (7): positive and negative carrier for IgG HBoV antibodies for genders.

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

Prevalence of HBoV- IgM HBoV for study samples

The results of the current study showed seropositivity IgM antibody test for infected children with HBoV at 7 cases and by (8.8%) with a significant difference when compared with control group (P<0.05) as shown in table (8).

Table. (8): Positive and negative cases of IgM HBoV antibodies.

Groups	Positive		Negative		Total		P Value
	No	%	No	%	No	%	
Patients	7	8.8	73	91.2	80	100	P=0.028
Control	0	0.0	10	100	10	10	
Total	7	7.8	83	92.2	90	100	

DISCUSSION

The prevalence of HBoV varies among different countries of the world due to climatic and geographical factors as well as health care or cultural and socioeconomic level, Several studies have indicated that HBoV prevalence is higher in winter.[10,11]

The results of our study showed that the rate of infection was (38.8%) by qualitative PCR. In previous study done performed by Atyah in 2017 in Iraq Showed HBoV infection in children 48/195 (24.62%) using Real Time PCR method.[12] A study performed in Egypt to detect the DNA of the virus in Nasopharyngeal swab in 2016 showed that the infection rate was (56.8%) for 95 samples.[13] A study performed in China in 2012 using Real Time PCR technology Showed the prevalence rate was (24.6%).[14] This difference can be explained by several factor causes including sampling techniques, study groups, climate variations and the sensitivity of several tests to detect the virus.[15] The results of our study demonstrated that the age group (3 months -1 years) recorded 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 lowest percentage was 28.5% in age group (8-10) years. A study performed by Atyah in 2017 in Iraq showed that the prevalence 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 appeared the age of children in the study (5) years was observed high incidence of HBoV in children aged (6 months - 1 year) 57.9%.[16] Another study appeared the rate of infection in the age group (6 months - 2 years) 63.91% .[17] Primary infection with HBoV occurs early in children between age (6 months - 2 years).[18] Newborns are less likely to have the infection because of protection antibodies obtained from mother.[19] The results of the this study in relation to gender showed that the rate of infection in males (40.0%), while the rate of infection in females (37.1%) and infection between males and females was Converged. The results of this study differ with study performed in Egypt appeared the infection of males consisted (78.86%), and showed a lower infections of females 21.14%[16] A study performed in China in 2016 appeared infections in males (65. 9%) while in female was (34.1%).[17]

Regarding IgG seropositivity outcome was (35%) with a significant difference when compared with control group. The results of this study corresponded to that performed in China by Lin *et al* which was (31%) of Children with lower respiratory tract infections. All age groups of children showed a higher level of antibodies to HBoV IgG using the ELISAs method and using human bocavirus VP2 virus-like particles.[20] While this study is not

compatible with a study performed in Finland that indicated IgG antibody was 111/258 (43%).[21]

The results of the current study showed that the age group (3 months -1 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 recorded (28.6%). No IgG was reported in the (8-10) year's age group. Seroepidemiological study in Jamaica in 2012 indicated that more than 80% of children are susceptible to HBoV at the age of two years.[22] Another study in Italy in 2012 indicated that the prevalence of IgG was 73.7% in children aged (1day to 5 months) and 51.4% in the (6-11) month age group, 64.2% in the (2- 4) years and the high rate of immunoglobulin IgG in the age group (5-9) years 96.4%.[23] The results of this study was showed the seropositive for IgG antibody among males (37.8%) while the rate of IgG in females (31.4%). The results of the current study in agreement with a study performed in Jamaica the rate of IgG infection in females (53.3%), which is higher than of males (46.7%).[22]

The results of this study was showed seropositivity IgM antibody test for infected children with HBoV (8.8%) with a significant difference when compared with control group. these results were agreement with study performed in Egypt in 2017 where the prevalence of IgM 16 / 123/ (13%) by ELISA.[16] As well as agreement with study performed in Finland was appeared IgM 16/ 121 (13.2% .)[24] As well as agreement with study performed in Germany in 2008 was showed immunoglobulin IgM 10/24 (42%).The differences may be due to social and environmental conditions, as well as the type of kit used for diagnosis and sample preparation for the infected patients.[25]

CONCLUSIONS

The study showed a high rate of HBoV infection in children with respiratory infections in Diyala province, The age groups (3months -1 years) had the highest incidence of infection and the lowest incidence was in the (8-10) age group using the PCR method. Using the ELISA technique the age group (3 months - 1year) reported high IgG infection and the lowest incidence was in the (2-4) age group. The incidence of infection between males and females is close using PCR method. Using the ELISA method an increase of HBoV IgG antibodies of males compared with females. The results of the study showed an increase in the number of patients with immunoglobulin IgG and IgM in patients with respiratory infections compared to healthy patients.

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