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Bocavirus and rotavirus co-infection in children with acute gastroenteritis and associated risk factors

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Abstract

Background: Both Viruses are the leading cause of acute gastroenteritis in infants and young children and are considered an important cause of mortality worldwide.

Objective: To estimate the rate of human bocavirus and rotavirus co-infection in children with acute gastroenteritis and determine any association with different risk factors.

Patients and Methods: Stool samples were collected from one hundred children suffering from acute gastroenteritis at the Central Teaching Hospital of Pediatrics, Baghdad, Iraq, for the period from December 2021 to April 2022. Samples were stored at -20 °C until they were utilized by PCR and ELISA to identify the human bocavirus (HBoV) DNA and rotavirus antigen (RV-Ag), respectively.

Results: This cross-sectional study showed that HBoV DNA was detected in 10 out of 100 samples (10%), while RV-Ag was detected in 22 out of 93 samples (23.66%), and the co-infection rate was 100% (All cases positive for HBoV were also positive for RV). None of the studied demographic data and risk factors showed a significant association with HBoV and/or rotavirus infection (P>0.05).

Conclusion: Most NICU deaths resulted from prematurity and respiratory distress syndrome. Enhancing healthcare personnel skills, standardizing protocols, and evidence-based practices for preterm and respiratory distress syndrome management can help reduce neonatal mortality rates in Iraq.

Keywords: Human bocavirus, rotavirus, acute gastroenteritis, risk factors, co-infection

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Introduction

Gastroenteritis is the second most common cause of mortality worldwide in children less than five years old [1]. Among their etiologic agents, viruses constitute 80% of cases [2]. Rotavirus (RV) is the leading cause of severe dehydrating gastroenteritis [3]. In 2009, the World Health Organization (WHO) recommended that all countries introduce

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rotavirus vaccines into their national immunization programs [4].

Human bocavirus (HBoV) was discovered in 2005 [5]. It is increasingly being identified as causative agents of diarrhea [1]. HBoV belongs to the genus Bocaparvovirus within the subfamily Parvovirinae and the family Parvoviridae. They are small, nonenveloped, icosahedral viruses with an approximately 5.3 kb single-stranded DNA genome containing three open reading frames (ORFs): the first ORF encodes nonstructural protein 1 (NS1), the second ORF encodes nuclear phosphoprotein (NP1), and the third ORF encodes the viral proteins VP1 and VP2. There are currently four bocavirus genotypes identified globally, namely human bocavirus genotypes 1 to 4 [6].

Studies have indicated that HBoV presence in patients with acute gastroenteritis ranges between 0.8% to 42% [7, 8]. On the other hand, HBoV was detected in 58% of examined cases in Egypt (1). Although bocaviruses have been associated mainly with respiratory diseases and gastroenteritis, recent reports have associated HBoV with heart disorders and encephalitis. The presence of small amounts of HBoV DNA has also been detected in healthy blood donors [9]. HBoV cases show a high rate of coinfection (up to 83%) with many viral and bacterial respiratory and gastroenteritis pathogens [10]. In 2015 study in Pakistan showed that 98% co infection between bocavirus and rotavirus [11]. The current study aimed to determine the rate of HBoV infection in children with acute gastroenteritis and also to determine the possibility of HBoV co-infections with rotavirus.

Patients and Methods

Subjects

In this cross sectional study, samples were taken from 100 infant and young children under the age of five years who had acute gastroenteritis from Central Teaching Hospital of Pediatrics, Baghdad, Iraq from December 2021 to April 2022. The mean age (13.77± 11.1 S.D. months) ranged from 1-40 month(s), 58 of them were boys and 42 were girls.

Samples collection: Little quantities of diarrheal feces were transferred to Eppendorf tubes using wooden sticks and disposable gloves, then the samples were labeled and transported to the laboratory at 4˚C, after that they were aliquoted to avoid repeated freezing and thawing and stored at -20˚C until further analysis.

Viral DNA extraction and amplification: DNA was extracted from 200 mg of stool sample by using the EasyPures Stool DNA Kit following the manufacturer instructions. A conventional PCR was performed to amplify the HBoV (1-4) VP1/VP2 according to (9), 453bp fragment was amplified by using Pan-Boca forward primer 4808 (5'-AAATGGCAAAAATTCCAGTTCC-3') and Pan-Boca reverse primer 5241 (5'-AAAACAGCTCCC CCCACAAT-3'). PCR reaction mix was performed in (25µl) total volume (5µl) template DNA, (1µl) of each forward and reverse primer, (5.5µl) Nuclease-free water and 12.5µl of PCR supermix. Then, thermal cycling was done as following: initial denaturation at 94ºC for 30 sec and 40 cycles of denaturation, annealing and extension at 94ºC, 56ºC and 72ºC for 5 sec, 30 sec and 30 sec, respectively, followed by final extension at 72ºC for 10 min. HBoV

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DNA positive control was provided by Dr. Arwa Al-Shuwaikh from previous study (12). While amplification mixture without the template was used as negative control, positive and negative controls were included with each run.

Detection of rotavirus antigen (RV-Ag): ELISA Kit (Sunlong Biotech Co., China) was used for the qualitative determination of RV-Ag in serum following the manufacturer instructions.

Statistical Analysis

Statistical package for social sciences (SPSS) 20.0 was used to conduct the statistical analysis. The mean and standard deviation were calculated to describe numerical data. Count and percentage were calculated to describe categorical data. Chi-square ($\chi 2$) test used to estimate the association between variables. The lower acceptable threshold for statistical significance is below or equal to 0.05.

Results

Ten samples were tested positive for HBoV DNA among children with acute gastroenteritis (10 out of 100). While 93 stool samples were screened by ELISA for the detection of RV-Ag, the results show that 22 out of the 93 samples (23.66%) tested positive for rotavirus antigen. This study showed that all the HBoV-positive infected children (n = 10) were co-infected with rotavirus (10 samples of the 22 positive RV-Ag were co-infected with human bocavirus), as shown in Table (1).According to sex distribution, of the 22 virally infected children, 14 are boys and 8 are girls. The age distribution of the viral positive cases revealed that (59.1%) were in the age group (1-13 months), (22.7%) were in the age group (14-26 months), and (18.2%) were in the age group (27-40 months). Approximately all virally positive cases (95.5%) were found in rural areas around the capital, Baghdad. Although the educational level of mothers did not appear to affect the rate of viral infection, 13 out of 22 (59.1%) of virally infected children had mixed feeding (breast and bottle) and 7 out of 22 (31.81%) of them consumed tap water as a source of water that was used to prepare their milk, as shown in Table 2. However, none of the above demographic and risk factors have a significant association with viral infection (HBoV and/or RV) ($P > 0.05$).

Table(1): Frequency of human bocavirus (HBoV) co-infection with rotavirus

HBoV DNA * RV-Ag Cross tabulation		RV-Ag		Total	Statistical analysis
		Positive	Negative		
HBoV DNA	Positive	10	0	10	χ^2 =36.16 P=<0.001* *
	Negative	12	71	83	
Total		22	71	93	

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Table (2): Distribution of HBoV and/or RV infection according to demographic data and risk factors in children with gastroenteritis

Characteristics		Viral infection (HBoVand/or RV		Total (n=93)	Statistical analysis*
		Positive No. (n=2	Negative No. (n=		
Sex	Boys	14 (63.6%)	37 (52.1%)	51(54.9)	χ^2= 0.9 P=0.432^{NS}
	Girls	8 (36.4%)	34 (47.9%)	42(45.1)	
Age group mont	1-13	13 (59.1%)	31 (43.7%)	44 (47.3%)	χ^2=2.18 P=0.338^{NS}
	14-26	5 (22.7%)	28 (39.4%)	33 (35.5%)	
	27-40	4 (18.2%)	12 (16.9%)	16 (17.2%)	
Residence	Rural	21(95.5%)	59 (83.1%)	80 (86%)	χ^2=2.1 P=0.144 ^{NS}
	Urban	1 (4.5%)	12 (16.9%)	13 (14%)	
Mother educatio	Illiterates	5 (22.73%)	13 (18.3%)	18 (19.4%)	χ^2=1.31 P=0.724 ^{NS}
	Primary	7 (31.81%)	16 (22.5%)	23 (24.7%)	
	Secondary	5 (22.73%)	21 (29.6%)	26 (27.95%)	
	Tertiary	5 (22.73%)	21 (29.6%)	26 (27.95%)	
Type of Feedir	Breast feeding	2 (9.1%)	20 (28.1%)	22 (23.6%)	χ^2=3.38 P=0.180^{NS}
	Bottle feeding	7 (31.8%)	18 (25.4%)	25 (26.9%)	
	Mixed feeding	13 (59.1%)	33 (46.5%)	46 (49.5%)	
Water source	Tap water	7 (31.81%)	24 (33.8%)	31 (33.3%)	χ^2=1.49 P=0.828^{NS}
	Filtered water	3 (13.63%)	9 (12.7%)	12 (12.9%)	
	Boiled filtered	5 (22.73%)	10 (14.1%)	15 (16.1%)	
	Bottled water	5 (22.73%)	16 (22.5%)	21 (22.6%)	
	Boiled bottled	2 (9.1%)	12 (16.9%)	14 (15.1%)	

*NS= none significant

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Discussion

The current study showed that all HBoV infected children are co-infected with RV (100%), as shown in table (1), these result are closely related with other study in Pakistan that showed 98% co-infection between bocavirus and rotavirus [7]. The observation of HBoV co-infection with different viruses is common [13, 14]. Therefore, there has been a debate regarding its pathogenicity [13]. Several studies in children with gastroenteritis showed an infectious rate of 8.1% in Germany [15], 8.5% in Taiwan [16], 8.7% in Turkey [17], 13% in Pakistan [7], 13.92% in Italy [18] and 14.4% in Iran [19]. Different factors might contributed to the variation in detection rate such as diagnostic technique used, the sample size and season during which samples were collected.

This study showed that RV-Ag was detected in 22 out of 93 (23.66%) of children under study. Other research performed locally showed that the rate of infection was 17% [20], 24% [21] in Baghdad province and 79.6% in Diyala-Iraq [22]. RV vaccine was reported to utilize in Iraq later in 2013 which contribute to reduce gastroenteritis caused by rotavirus [20]. A study carried by Abdulazeez et al, (2002) demonstrated that RV positivity represented the higher rate among other viruses (i.e. sapovirus and norovirus) and all other detected viruses had concurrent RV infection [22]. Prevalence of infectious diarrhea influenced by socioeconomic status i.e. nutrition, sanitation and habitat of the population [23].

The current study showed that there was no significant association between viral infection and sex, age, residency, mother education level, type of feeding and source of water that used to prepare milk, as shown in Table (2). This results consistence with [22], who reported that there was no significant association between RV infection and sex, mother education and water sources but a significant association was found between RV positivity and age. In addition, another study reported that no sex preference to acquired viral infection but RV-Ag was detected more in children less than 2 years of age [21]. Although this study showed there was no significant association with age, viral infection was showed higher percentage in age group 1-13 month(s) (59.1%), this consistent with other who reported that the age group 7-11 months represent the peak incidence of rotavirus [23].

The current study conducted on stool samples taken from central teaching hospital of pediatrics in Baghdad province, however, the majority of children from rural area around Baghdad rather than the city itself. and 21 out of 22 (95.5%) of viral infected children from rural area, this results are in agreement with study that done in South Africa [6] and study in Baghdad about other enteric viruses [24]. A previous study showed that maternal nutrition knowledge is essential for preventing disease and parents with lower education levels are more likely to have inadequate nutritional understanding [25]. Another study showed RV-Ag was detected more in children with low mother education and rural patients and less among breast feeding children [21]. Al Kerwi et al. (1993) mentioned that socioeconomic status such as education level, household type, water source, and toilet might be a risk factors for RV infection. Indoor toilets were significantly associated with RV infection,

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but were independent of other factors. However, other studies have not demonstrated a role for socioeconomic factors or sanitation, except for overcrowding [26].

The result of this study showed the viral infections were not associated with the type of feeding, however the highest percentage of viral infection was showed in children with mixed feeding (59.1%), followed by bottle feeding (31.8%), while the lower percentage of viral infection was showed in children with breast feeding (9.1%). Breastfeeding protects against diarrhea of all causes in infants, however, there is no evidence that breastfeeding provides specific protection against viral gastrointestinal infections and it was failed to prevent rotavirus infection or reducing the severity of symptoms of rotavirus infection [23].

The higher viral infection in children drink milk prepared with tap water (31.81%) comparing with bottled, filtered and boiled water could explained by the fact that filtration system not sterilizes water due to the filters might not have been changed for a long time, in addition, boiling water might not get reach the temperature that high enough to kill the microorganisms. In addition, the wastewater treatment systems handle a wide range of viruses. More than 140 different types of enteric viruses have been identified in human stool. Public health information indicates that these types can lead to a wide range of water related diseases [27].

Conclusions

Higher rate of RV infection was found in infant and young children with acute gastroenteritis especially those from rural areas.

Recommendations

The health care authorities must build up an improved system for children's RV immunization as a mandatory preventive measure. Additional research with larger sample size is needed to examine the role of bocavirus in the pathogenesis of acute gastroenteritis.

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Ethical clearance: Ethical approval was obtained from the Institutional Review Board (IRB) of College of Medicine, Al-Nahrain University (No.20211160 in 5th December, 2021).

Conflict of interest: Nil

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العدوى المشتركة بين فايروس البوكا وفايروس العجلية عند الأطفال المصابين بالتهاب المعدة والأمعاء الحاد وعوامل الخطر المرتبطة به

محمد ياسين محمد [١] ، أروى مجاهد الشويخ [٢] ، حيدر طالب الحمداني [٣]

الملخص

خلفية الدراسة: الفايروسات هي السبب الرئيسي لالتهاب المعدة والأمعاء الحاد عند الرضع والأطفال الصغار وتعتبر سببًا مهمًا للوفيات في جميع أنحاء العالم.

اهداف الدراسة: لتقدير معدل العدوى البشرية بفايروس بوكا والفايروس العجلية (فايروس الروتا) عند الأطفال المصابين بالتهاب المعدة والأمعاء الحاد وتحديد أي ارتباط بعوامل الخطر المختلفة.

المرضى والطرائق: تم جمع عينات البراز من مائة طفل يعانون من التهاب المعدة والأمعاء الحاد من مستشفى الطفل المركزي التعليمي ، بغداد ، العراق ، للفترة من كانون الأول ٢٠٢١ إلى نيسان ٢٠٢٢. تم تخزين العينات في درجة حرارة -٢٠ درجة مئوية حتى تم استخدامها بواسطة تفاعل البوليمراز المتسلسل (PCR) ومقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) لتحديد الحمض النووي لفايروس بوكا البشري (HBoV) ومستضد فايروس الروتا (RV-Ag) ، على التوالي.

النتائج: تم الكشف في هذه الدراسة المقطعية عن HBoV DNA في ١٠ من أصل ١٠٠ عينة (١٠٪) ، بينما تم الكشف عن RV-Ag في ٢٢ من أصل ٩٣ عينة (٢٣,٦٦٪) ، وكان معدل الإصابة المشتركة ١٠٠٪ . لم تظهر أي من البيانات الديموغرافية وعوامل الخطر ارتباطًا ذات دلالة احصائية مع الاصابة الفايروسية ($P > 0.05$).

الاستنتاجات: جميع الحالات الإيجابية لـفايروس البوكا HBoV كانت إيجابية أيضًا لفايروس الروتا RV لأن كلا الفايروسين يشتركان في نفس طرق الانتقال أو لأن RV يمهد الطريق لعدوى البوكا أو إعادة تنشيطه. كما يجب تطبيق برامج تحصين أكثر تقييدًا لفيروس الروتا في المناطق الريفية.

الكلمات المفتاحية: فايروس البوكا البشري ، فايروس العجلية ، التهاب المعدة والأمعاء الحاد ، عوامل الخطر ، العدوى المشتركة

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