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WHOLE-GENOME SEQUENCING ANALYSIS OF HUMAN BOCAVIRUS DETECTED IN CHILDREN SUFFERING FROM RESPIRATORY TRACT INFECTION IN BASRAH CITY, SOUTH IRAQ

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Abstract
In 2005, Allander and colleagues at Karolinska Institute found the human bocavirus (HBoV) in a collection of nasopharyngeal aspirates. There are four *human bocavirus* species, HBoV1-4 the three species HBoV2, HBoV3, and HBoV4 were caused gastroenteritis and mainly found in stool samples. While HBoV1 causes upper and lower respiratory tract infections (RTI). The HBoV genome is arranged into three open reading structures, ORF1 at the 5' end, ORF2 at the 3' end, and ORF3 at the 3' end, encoding two protein structures, VP1 and VP2. Two hundred oropharyngeal swab specimens were collected from hospitalized children under 5 years old who have been suffering from acute respiratory tract infection (ARTI) from Basrah Maternity and Children Hospital in Basrah city. In the period from December 2020 to December 2021. The human bocavirus was identified by Multiplex OneStep. In a total of 200 tested samples, HBoV was detected in 28 (14%) samples. Analysis of the whole sequence of strain NHAB1 (OP593329) revealed that this strain is similar to HBoV strains at 98.14–98.72%. The allusion strain HZ1303 (KP710210) discovered in China had the highest identical sequences (98.72%). The current study isolates clustered with KP710210 (HBoV type I China isolation). The nucleotide sequences of these genes (NS1, NP1, VP1, and VP2) were translated into the corresponding amino acid. It was discovered that the NS1 aa contained three aa substitutions: at position aa106, glycine (Gly) changed to arginine (Arg). Also, in position aa228 the cysteine (Cys) substituted to glycine (Gly) and in position aa229 the asparagine (Asp) substituted with glutamine (Glu). One aa alteration was found in the NP1 aa sequences at position aa79, where serine (Ser) was changed to asparagine (Asn). While, Among VP1 and VP2 aa there was not substitution detected.

Keyword: HBOV, ARTI, genome, sequencing

1. Introduction

The *human bocavirus* (HBoV) was discovered by Allander and coworkers at Karolinska instituted in 2005 in pooled nasopharyngeal aspirates (Allander *et al.*, 2005). Following the phylogenetic investigation of the HBoV genome, its name was coined, showing that it shared a close relationship with the canine MVC and the bovine parvovirus (BPV1) (Zhou et al., 2014). There are four human bocavirus species, designated as HBoV1-4, which are members of the Parvoviridae family, Parvovirinae subfamily, and Bocavirus genus. HBoV2, HBoV3, and HBoV4 were three species that primarily appeared in stool samples and caused gastroenteritis. While lower and upper respiratory

tract infections (RTI) caused by HBoV1 (Yaseen *et al.,* 2020). HBoV is a small (18-26 nanometers), non-enveloped linear single-stranded DNA, icosahedral virus (Huang *et al.,* 2012). The HBoV chromosome is about 5.3 kb in size and is organized into three open reading frames, with ORF1 at the 5' end translating the nonstructural peptide NS1, ORF2 at the 3' end expressing the nonstructural protein NP1, and ORF3 at the 3' end coding two structural proteins, called VP1 and VP2 (Watanabe et al., 2018). In children less than 5 years of age the HBoV was more commonly, which causes respiratory tract infections leading to hospitalization in this group (Bastien *et al.,* 2006; Maggi *et al.,* 2007).

Worldwide, HBoV1 consider the fourth most prevalent respiratory virus. This virus was detected in children with respiratory tract infections their age less than two years old with an epidemiology range of 2-33% (Atyah *et al.,* 2017). The most frequent clinical diagnoses linked with HBoV1 include upper respiratory tract infections (RTI), bronchiolitis, pneumonia, bronchitis, asthma exacerbation, and pharyngitis. Noor et al. (2017); Abdel-Moneim et al. (2016). This study's objectives are to estimate the prevalence of HBoV infection and pinpoint the genotype among young children in Basrah Cyyy who have respiratory tract illnesses.

2. Materials and methods

2.1. Patients and samples collection

A total of two hundred oropharyngeal swab specimens were collected from hospitalized children under 5 years old who have been suffering from acute respiratory tract infection (ARTI). Samples were collected using VTM (viral transport media), from Basrah Maternity and Children Hospital in Basrah city. In the period from December 2020 to December 2021.

2.2. Detection of *Human Bocavirus*

Viral nucleic acid was extracted from 200 oropharyngeal swabs using QiaAmp Viral Mini Kit (Qiagen) and prepared based on the manufacturer's protocol. After the extraction of viral nucleic acid from samples, the HBoV were identified by Multiplex OneStep by using DiaPlexC^{TM} RV13 Detection Kit of SolGent company. This kit provides the detection of a group of respiratory viruses in which HBoV one of the included viruses. The reaction constituents are prepared based on the manufacturer's protocol. The conditions of the thermocycler were set based on the kit program. The PCR product of the HBoV was determined by using 3% agarose gel electrophoresis at 347 bp.

2.3. *Human Bocavirus* whole genome sequencing

Samples were amplified by using 13 pair of primers. The primers designed using the Fast PCR according to the full length of the HBoV reference strain genome the HZ1303 (KP710210) and sequencing the positive results by sanger method. The National Institute for Biological Information's (NCBI) BLAST program was used to examine the entire sequencing genome findings to identify the HBoV genotype.

2.4. Phylogenetic analysis

Mechanical Evolutionary Genetic analyses (MEGA) software version 11.1 was used to compare sequence analyses, including sequence aligning and genetic distance calculation (Tamura et al., 2021). Branch stability was assessed based on 1000 bootstrap repeats and trees of phylogeny were built using the neighbor-joining approach with a Kimura two factor model in MEGA (Saitou and Nei, 1987).

2.5. Amino acids sequences analysis of HBoV genes

Sets of protein amino acids have been generated by performing forward and opposite transcriptions of DNA sequences to amino acidity using the Open Reading Frame (ORF) program of any sequence in this study using the NCBI- ORF online database. This process began with the trimming and

confirmation of nucleotide sequence sets of NS, NP, VP1, and VP2 genes with other world DNA sequences using NCBI- Blast.

3. Results

3.1. Frequency of Human Bocavirus

In a total of 200 tested samples, HBoV was detected in 28 (14%) samples as shown in Figure (1).

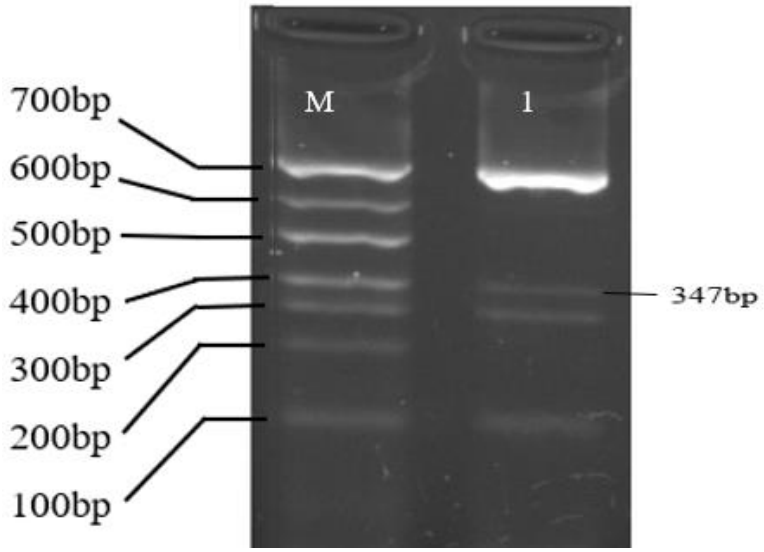

Figure (1): Agarose gel electrophoresis construction of the PCR product of HBoV. Lane M, ladder (marker) 1000-100bp. Lane 1, positive sample for HBoV= 347 bp. Agarose 3%, 100v for 30 min.

3.2. Human Bocavirus whole genome sequencing

In this study, 13 primer pairs were designed which depended on reference strain HZ1303 (KP710210) from China for amplification the sample. The PCR product of positive HBoV sample has been successfully sequenced. The whole sequences of nucleotides obtained from Iraq strain NHAB1 (OP593329), were matched with those of recognized source varieties of HBoV to know the genotype of the newly detected strain. The length of the whole sequences genome of Iraq strain NHAB1 (OP593329) is 5297 bp. Analysis of the whole sequence of strain NHAB1 (OP593329) revealed that this strain is similar to HBoV strains at 98.14–98.72%. Table (1) shows that the greatest level of sequence identity (98.72%) was observed with the Chinese reference strain HZ1303 (KP710210).

Table (1): Comparison of whole sequences of nucleotides obtained from Iraq strain NHAB1 (OP593329) with other reference strains.

Reference strain	Accession number	Country	Nt sequence identity (%)
HZ1303	KP710210	China	98.72
CQ201101.2	JN387083	China	98.61
Fukushima/OR331/2021 DNA	LC720422	Japan	98.59
HBoV/YOK /08/104	AB551032	Japan	98.59
xz040-boca1	OL519573	China	98.57
Parvozj-6	MZ546191	China	98.55
CRD2	DQ340570	USA	98.55
NB1621741	MK034749	Netherlands	98.55
TUN2207	JF327786	Tunisia	98.54
CU6	EF203920	Thailand	98.54
BJ3064	DQ988933	China	98.46
Mty1117	KX373885	Mexico	98.44
JPOC08-011	AB481081	Japan	98.37
TW265_07	EU984243	Taiwan	98.33
HK9	EF450725	HongKong	98.14

3.3. Phylogenetic analysis of *human Bocavirus*

Two distinct groups emerged throughout the phylogenetic study. The sequence acquired in the present research and the HBoV-1 genotype reference sequences retrieved from GenBank figure (2) formed the first major branch of the tree, which was then subdivided into two sub-branches. GenBank now has the sequences produced for this investigation under the accession numbers OP593329. MEGA 11.1 software was used to construct a restricted phylogenetic tree (Figure 4) to examine the genetic relatedness of the local isolate. The local isolate is clustered with the reference isolate belonging to HBoV type 1. The current study isolates clustered with KP710210 (HBoV type I China isolation).

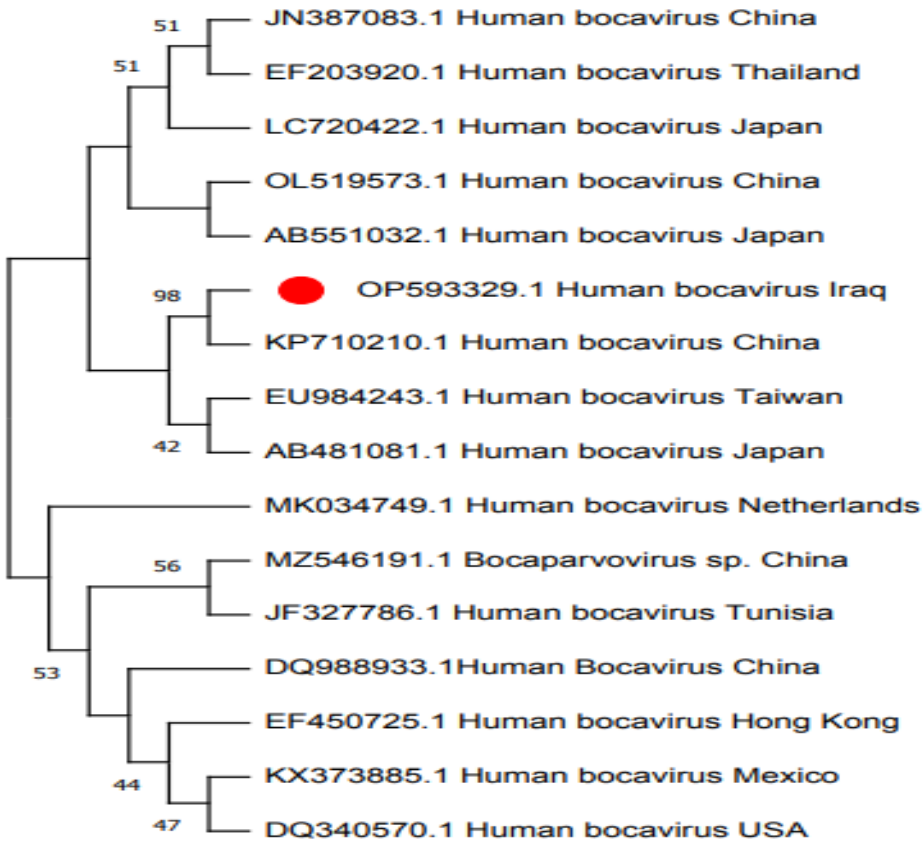

Figure (2): Human Bocavirus phylogenetic analysis using complete genome sequences. Mega 11.1 software was used to create phylogenetic trees with 1000 bootstrap replicates using the neighbor-joining method. For the genotype analysis of OP593329 (red circle), reference strains of HBoV were utilized. The HBoV strain (OP593329) obtained for this study was ultimately recognized as HBoV1.

3.4. Amino acid sequence identities of *human bocavirus*

The nucleotide sequences of these genes (NS1, NP1, VP1, and VP2) were translated into the corresponding amino acid. However, the NS1 gene of strain NHAB1 encodes a polypeptide of 399 aa remains, the NP1 gene encodes a polypeptide of 220 aa remains, the VP1 gene encodes 265 aa residues and the VP2 gene which encodes a polypeptide of 542 aa remains. As well, the different amino acid sequences for these genes of isolate NHAB1 were aligned and compared with different amino acid sequences of reference sequences. According to the results of the alignment found the NS1 aa There were 3 aa changes found: in position aa106 in which glycine (Gly) change to arginine (Arg). Also, in position aa228 the cysteine (Cys) substituted to glycine (Gly) and in position aa229 the asparagine (Asp) substituted with glutamine (Glu). One aa substitution was found in NP1 aa sequences in aa79 the serine (Ser) substituted with asparagine (Asn) as shown in figure (3). While, Among VP1 and VP2 aa there was not substitution detected.

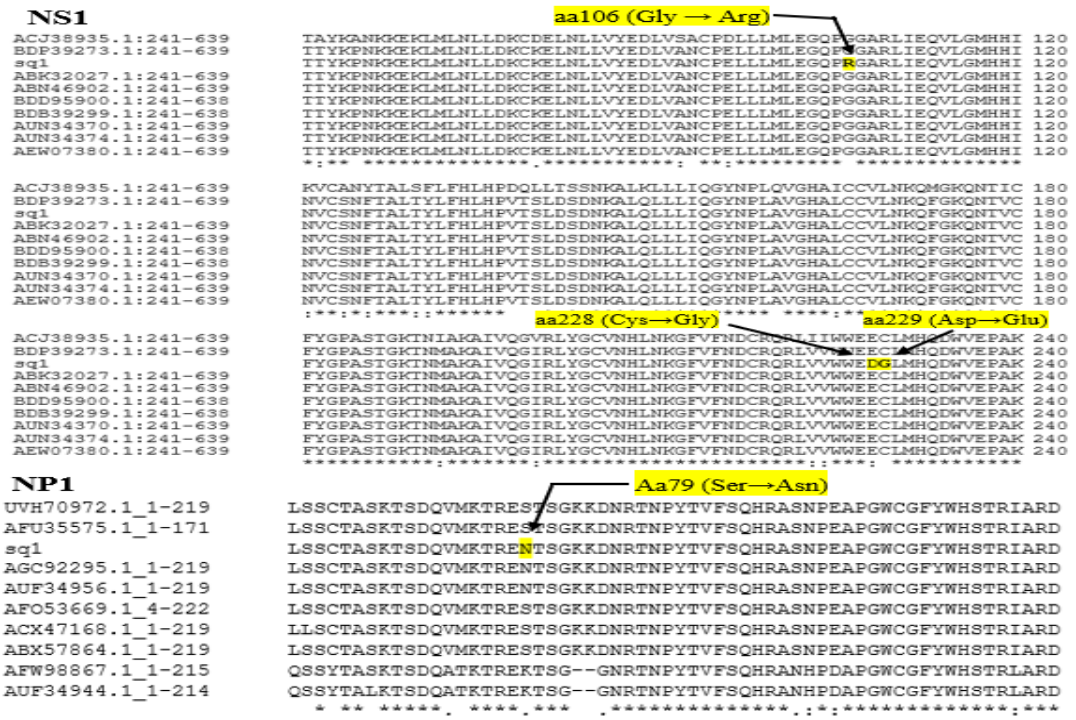

Figure (3): Comparison of NS and NP1 amino acid substitutions among refence strains for HBoV genotype. The yellow highlighted indicate the sites of amino acid alterations.

5. Discussion

Respiratory viral infection is crucial in public health due to its ease of spread (Dawood *et al.*, 2012). Pediatrics needs special care in drug administration because even minor errors in dosage or duration of medication use can have serious consequences (Mohammed-Jawad *et al.*, 2020). Therefore, sensitive and effective methods of detection of these agents as multiplex PCR assay are necessary to properly treat patients and prevent the use of unnecessary antibiotics in hospitals specifically inpatient pediatric age groups (Thurman *et al.*, 2009; Hussein, 2018).

Our study retrospectively assessed the respiratory tract viruses detected in two hundred hospitalized children with symptoms of respiratory tract infections and their age under five years old. During the study period the molecular assays were used . In our study, the HBoV was detected with rate (14%) among hospitalized children with respiratory infections. This rate lower than reported by Yaseen *et al*. (2020) found HBoV infection is (38.3%), but higher than another study from India with rate (6.6%) by Bharaj et al. (2010), and in Iran with rate (13.3%) in a study by Hosseininasab et al. (2021). In this study, to obtain the whole sequence of viral genome for HBoV-positive specimens the traditional method, Sanger sequencing was used. However, it was difficult to obtain complete sequencing because of the low viral load. Further, the process was labor-intensive and time-consuming. Thus, to amplify the whole genomes of the HBoV-positive specimen, we designed 13 sets of universal primers (by using reference strain from GenBankThe prevalence and genetic features of HBoV are also poorly understood in Basrah city. This work, using a strain recovered from a 2-year-old male infant with acute respiratory tract infection, is the first to identify the full genome sequencing of HBoV in Basrah city. As stated to phylogenetic analysis of HBoV, after the alignment of this local isolate sequence with 16 references isolates by MEGA 11.1 software, found the local isolate was not identical (100%) to any references strain but the compatibility was 98.14–98.72% with dissimilar references strain. Possible explanation for less-than-complete identity: this is the first research in Basrah city to employ whole sequences. Recent research conducted by Kang et al. (2018) found that there was less than 100% identity among Gene Bank reference infections and Korean HBoV isolates, with similarity ranging from 98.77 to 99.5%. According to a separate Iranian research by Nadji et al. (2010), the level

of sequence similarity between Iranian HBoV isolates and Gene Bank isolates was between 98.95 and 99.88%. It was shown in a research conducted by Hasan et al. (2020) in Baghdad, Iraq, that although the isolates were not genetically identical to any reference isolate, they were 99.9 percent genetically compatible with a variety of reference strains. Also, in the current study the phylogenetic analysis for this isolate was found to be related to HBoV type 1, the strain NHAB1(OP593329) is very similar to the China HBoV type I isolate (KP710210) with a rate of 98,72% which was reported from a child with acute respiratory tract infection at china (GenBank). The findings of this investigation were consistent with those of many other studies conducted in nearby nations. In Saudi Arabia study by Abdel-Moneim *et al*. (2013) reported only HBoV type 1 infection from 80 children with RTI. Similarly, the study by Abozahra *et al*. (2020) detected the HBoV-1 genotype only among children suffering from respiratory tract infections. The sole type 1 HBoV was found in this international investigation of children with acute respiratory illness (Kenmoe et al., 2017). However, only HBoV1 was found in children with acute respiratory infections in Vietnam (Jartti *et al.,* 2012). On the other hand, in Japan, Koseki *et al*. (2012) detected all four HBoV genotypes (HBoV 1–4) in nasopharyngeal samples collected from children with respiratory tract infections. This suggests that HBoV1 may be one of the common pathogens in children's hospitalizations for acute respiratory tract infections. As mentioned earlier our strain was close to the China reference strain, the reason for close to China isolation may be due to the number of a visitor during trade exchange or religious tourism. The existence of Iraq's isolation may be brought on by foreign tourists who visit the country for tourism, or perhaps all these isolates are global in nature. The local isolate sequence alignment with 9 reference sequences in the current study corresponding amino acid sequences between local and reference strains for (NS1, NP1, VP1, and VP2) amino acid. The amino acids sequence varies in a sequence of amino acid, especially among NS1 amino acid which had two substitutions of amino acid. While NP1 amino acid had only one substitution of amino acid. Likewise, about the VP1/VP2 both of them had no variant of the amino acid sequence of the local isolate. Numerous studies had nucleotide and amino acid sequence diversity, which was important for the current study's analysis of amino acid substitution. A study in Iraq by Hasan *et al*. (2020) found some variants in amino acids noticed deletion of amino acids occurred in more than two positions but the substitution was revealed in three positions of the VP1/VP2 amino acid sequence. Of note, Al-Shuwaikh (2021) found the NP1 amino acids sequence alignment of the sequenced sample showed when compared to the referring HBoV sequences, the analyzed specimen showed no mutations. A Chinese study by Wang *et al*. (2016) represented the screening of 993 respiratory samples for HBoV by PCR revealed that the evolutionary substitutions were visible in NS1. Novel molecular signatures allowing subtype differentiation between HBoVs were created by the nucleotide deletions and substitutions that occurred in NP1 and VP1. Seven of the 13 strains in a different Italian study on sick children with respiratory diseases had a mutation at one of the VP1/2 gene's three known sites (Principi *et al.,* 2015). A mutation at a specific site, such as a conserved residue, can change both the protein's structure and function. However, a mutation that alters the amino acid sequence may change a protein's structure without necessarily altering its function (Prabantu *et al.,* 2021).

6. Inconclusion

HBoV1 may be one of widely observed pathogens in children with acute respiratory tract infections. The local isolate was close to Chinese isolate. The aa substitutions were detected; three in NS1and one in NP1. While among VP1 and VP2 there was not aa substitution.

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