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Original Article

Detection of Human Boca Virus in Gastric Adenocarcinoma

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Abstract

Background: Gastric cancer is one of the most common cancers worldwide. Human bocavirus (HBoV), a recently isolated virus, has been investigated for its role in many respiratory and enteric diseases. Few studies have reported its presence in solid tumors, such as lung and colon cancers. The aim of this study was to detect the presence of the HBoV1 genome in gastric adenocarcinoma, which has not yet been evaluated.

Methods: Formalin-fixed paraffin-embedded (FFPE) blocks of 189 gastric tumors and 50 blocks of non-tumor gastric tissue products from elective weight reduction operations were collected. DNA extraction and real-time polymerase chain reaction (PCR) were performed for HBoV1 detection. DNA sequencing was performed using ABI Genetic Analyzer series 3500.

Results: The mean age of the patients was 60±13.33 years. Tumors were more common in males than females (2.5/1). HBoV1 PCR was positive in 34 (18%) cases of GC and 10 (20%) cases of chronic gastritis ($P>0.05$). There was no association between age, sex, stage, and histologic subtype of the tumor and HBoV1 positivity ($P>0.05$) in tumor samples. The rate of intestinal metaplasia and presence of lymphoid stroma were also not more frequent in HBoV1-positive tumors ($P>0.05$).

Conclusion: The HBoV1 can be detected in a relatively high proportion of Iranian patients with gastric cancer (18%) with no predilection for specific subtypes and no association with the degree of lymphocytic infiltration. HBoV1 can also be observed in approximately 20% of chronic gastritis cases. Further comprehensive studies are needed to elucidate the role of HBoV1 in gastric cancer development.

Keywords: Human Boca virus, Gastric adenocarcinoma, Real-time polymerase chain reaction

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Introduction

Gastric cancer (GC) is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related death worldwide. Among men, it is the leading cause of cancer-related deaths in Iran.[1]

The pathogenesis of this cancer is complex and involves many contributing factors.[2] One of the environmental factors in the development and progression of some cancers is microbial infection, which can be potentially preventable and treatable. *Helicobacter pylori* is a well-recognized infectious agent associated with gastric carcinogenesis. It is estimated that 50% of the global population is infected with *H. pylori*, and about 1%-3% of them develop GC.[3] Fortunately, the successful eradication of *H. pylori* infection is associated with decreased risk of GC.[4] Epstein-Barr virus (EBV) is another possible infective agent which has been estimated to be responsible for 5.6 –19.5% of cases with GC globally.[5] However, the potential carcinogenesis of some viruses other than EBV that are associated with GC risk remains unclear. A potential association between the hepatitis B virus (HBV) and the risk of GC was suggested in one case-control study.[6] It has also been reported that human cytomegalovirus infection may contribute to gastritis and be an underlying etiology of GC.[7] The role of HPV in GC has also been proposed but has not been documented in recent studies.[8–11]

Recently discovered human bocavirus (HBoV), a single-stranded DNA virus, has been reported in respiratory and gastrointestinal diseases. There is increasing evidence that the widespread HBoV plays a significant role in respiratory tract infections (RTIs).[12-15] HBoV is a member of the genus Bocaparvovirus, subfamily Parvovirinae, and family Parvoviridae, which contains four genotypes (HBoV1–4). The disease course of HBOV results from initial infection of the airway, followed by infection of the gastrointestinal tract, and subsequent shedding via the

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respiratory and rectal routes.[16]

HBoV is assumed to be the second parvovirus that causes various human diseases, such as RTI,[17] childhood gastroenteritis, myocarditis,[18] and encephalitis.[19] However, its role in gastrointestinal infections is now doubtful, based on a novel study conducted in the UK.[20]

Its classification as a bocavirus is based on similarities in the genetic structures and amino acid sequences with those of bovine parvovirus and canine minute virus.[21,22] Molecular biology studies have revealed that HBoV contains a 5.2-kb single-stranded DNA genome without an envelope.[23] Parvovirus B19 was the first virus from the Parvoviridae family and has been associated with several cancers, including lymphomas,[24] testicular tumors,[25] papillary and anaplastic thyroid carcinomas.[26,27] Some parvoviruses have been suggested to integrate into the host genome.[28] The disease course of HBoV results from initial infection of the airway, followed by infection of the gastrointestinal tract and subsequent shedding via respiratory and rectal routes.[16]

The hypothesis that HBoV can persist in the stomach, cause long-term damage, and prepare a microenvironment for cancer development is a plausible theory that should be verified. As an initial study, we aimed to assess the presence of the HBoV1 DNA genome in GCs with different histologies as well as in non-tumoral gastrectomy samples as a control group.

Materials and Methods

Sample Selection

This study was performed retrospectively. Paraffin blocks of 189 gastrectomy cases from different geographic regions of the Islamic Republic of Iran were included in the study. Patient age, sex, anatomical site, and histological classification were retrieved from hospital charts. 50 paraffin block samples, which were products of a gastric sleeve operation as an elective weight reduction procedure, were used as controls. These samples were evaluated using the Sydney system for gastritis evaluation.

Real-Time Polymerase Chain Reaction

Selected paraffin blocks of the tumor specimens were reviewed to obtain adequate tumor tissue and less normal tissue. Nucleic acids were extracted from six 5-µm slices of paraffin blocks. The DNA extraction was performed using the instruction of a formalin-fixed paraffin-embedded (FFPE) tissue kit extraction (Roje Technologies Company, Yazd, Iran). Real-time polymerase chain reaction (PCR) was performed using a primer-probe-based kit that detects the HBoV-1 genome (Genesig standard kit, Genesig, Primer design Ltd., Southampton, UK). The PCR was performed in a final volume of 20 µL. For the first tube, each microtube contained 5 µL of the tumor DNA or control, which was mixed with 10 µL 2X qPCR Master Mix, 1 µL HBoV primer/probe mix, 1 µL internal control, primer/probe mix, and 1 µL RNase/DNase-free water. For the second endogenous control, each microtube contained 5 µL of tumor DNA, which was mixed with 10 µL 2X qPCR Master Mix, 1 µL endogenous control primer/probe mix, and 4 µL RNase/DNase free water. The reaction tubes were then placed in a real-time PCR machine (Rotor-Gene 6000). The procedure consisted of an initial denaturation step at 95 °C for 2 minutes, followed by 50 cycles of 10 seconds at 95 °C, and 60 seconds at 60 °C.

PCR Product Purification and Cycle Sequencing

The PCR products were purified using the ethanol/sodium acetate purification method. Then, 0.5 µL purified PCR product was added to 2.5 µL, 0.5 µL BigDye, 0.5 µL primer, and 16 µL distilled water. Thereafter, Cycle sequencing was performed under the following thermal conditions: initial denaturation at 96°C for 1 minute, followed by 25 cycles at 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 minutes. Previously designed primers for *HBoV* type 1 (Forward GGCAGAATTCAGCCATACTCAAA and reverse TCTGGGTTAGTGCAAACCATGA) were used for Sanger sequencing of positive cases. An ABI Genetic Analyzer series 3500 (ABI, Foster City, California) was used for Sanger sequencing. Chromas bioinformatics software was utilized for trimming the raw data, and alignment of the consensus sequences.

Statistics

Statistical analyses were performed using SPSS software for Windows, version 20. Data are presented as mean ± standard deviation (SD) or number and percentage. The chi-square test was used to evaluate the statistical association between GC Bocavirus status and clinicopathological characteristics. Statistical significance was set at $P \leq 0.05$.

Results

The mean age of the patients was 60 ± 13.33 years, ranging 20-90, and the median was 60 years. Tumors were more common in males than females (2.5/1). According to the Lauren classification, 108 cases were intestinal type (57.1%), 60 were diffuse type (31.7%), 21 were mixed type (11.1%).

According to the WHO classification, the tumors were classified as 78 tubular (41.3%), 17 papillary (9%), 66 poorly cohesive (34.9%), 6 mucinous (3.2%), 21 mixed gastric carcinomas (11.1%), and 1 neuroendocrine carcinoma (0.5 %). The total number of carcinomas containing lymphoid stroma was 42 cases (22.2%) consisting of minimal 17 (9%) and moderate to severe 25 (13.2%). We regarded cases with moderate to severe lymphoid infiltration as medullary-type carcinoma.

Thirty-four patients (18%) were positive for the bocavirus, type 1. Evaluation of the sequence result with BLAST led to 98% identity with the HBoV, especially those strains registered in Iraq and Riyadh (Figure 1).

In HBoV1-positive cases, the mean age was 55.39 ± 16.91 years, ranging 20-82 and the median age was

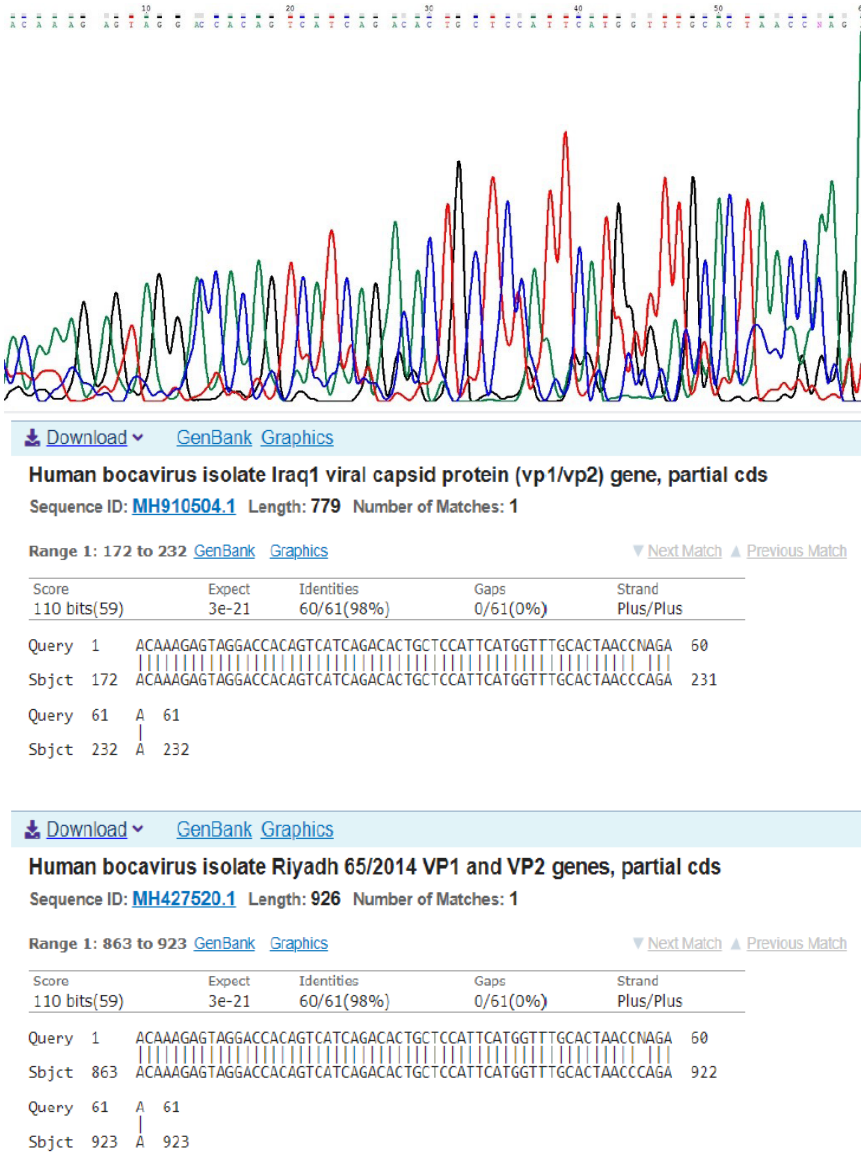

Figure 1. Chromatogram & initial trimming result with nucleotide BLAST showed 98% identity with human Boca virus especially those strains registered in Iraq and Riyadh

60 years, which was more common in males than females (m/f = 3.3). According to the Lauren classification, 22 (64.7%) Boca virus-positive cases were intestinal type, 8 (23.5%) were diffuse type, and 4 (11.8%) were mixed type, showing no significant predilection to a specific type different from the original tumor population ($P = 0.519$). According to the WHO classification, 17 were tubular (50%), nine were non-cohesive (26.5%), four were papillary (11.8%), three were mixed (8.8%), and one was mucinous (2.9%) ($P = 0.793$). In Boca virus-positive cases, the number of medullary carcinomas or carcinomas with intense lymphoid infiltration in the stroma was five out of 34 cases (14.7%) ($P = 0.48$).

There was neither association between the positivity of HBoV1 with intestinal metaplasia nor with gastric dysplasia in the territory of tumor samples. Of the 34 positive HBoV1 cases, 2 were positive, and 32 (94.1%) were negative for EBV. Of 155 that were negative for HBoV1, 5 were positive, and 150 (96.8%) were negative for EBV ($P = 0.458$).

Among the non-tumor gastric samples, 18 showed moderate to severe chronic gastritis, 24 had mild gastritis, and seven were normal. A total of 10 samples (20%) were HBoV1 positive, showing no difference from tumor samples ($P > 0.05$). Histopathological evaluation of the samples showed three samples with moderate-to-severe chronic gastritis and seven with mild chronic gastritis; among these, only one had *H pylori* at the surface of the epithelium.

Discussion

In this study, we evaluated 189 GC cases and 50 non-GC controls for detection of the HBoV1 genome in their gastric tissue using a real-time PCR assay. The Results showed that 34 (18%) samples in the cases and 10 (20%) in the control groups contained the HBoV1 genome. Evaluation of the sequenced PCR product showed 98% identity with HBoV strains registered in Iraq and Riyadh.

Recent studies have shown a relationship between HBoV and some diseases of the gastrointestinal and respiratory systems. The potential of HBoV for persistent infection by possibly integrating the viral genome into the host chromosome raises concerns about its conceivable role in cancer production. Like other oncogenic viruses, HBoV genome establishes a persistent infection in infected cells as covalently closed circular DNA (cccDNA), but it is not known whether it integrates into tumor cells or remains episomal.[29] Some reports suggest that closed circular DNA formation occurs during HBoV DNA replication, and its episomal form can exist in continuous infection.[30] It is hypostatized that HBoV can replicate in dividing and proliferating cancer cells.[16,31]

HBoV-1 has been primarily reported in respiratory tract specimens, feces, plasma/serum, and possibly lymphoid tissue samples where persistent infections can frequently occur.[32] As a primary study in Iran, we found that there were 18.4% (44/239) HBoV1 genomes in gastric tissue which could indicate its possible role in the further development of cancer, although we did not find significant results. This may be due to the low prevalence of viruses in our community or the limited sample size.

There are some reports of HBoV infection in stool and respiratory samples from the Iranian population, but few studies have been conducted on other samples.[17,33] In this study, we attempt to use gastric tissue samples for a primary study in Iran. Naghipour et al[34] first reported HBoV in 8% (21/261) of Iranian children with acute respiratory infections. Nadji et al[17] in Iran used respiratory and stool samples from children with acute respiratory tract diseases and gastroenteritis for the detection of HBoV (NS-1) gene via PCR method. They reported that 6.8% (9/133) of the respiratory samples and 12.8% (6/47) of the stool samples were HBoV-positive.

Karbalaie Niya et al[33] showed that such a high incidence of HBoV was not present in Iranian cases of colorectal cancers. They found that only one of 66 examined colorectal cancers was positive for the HBoV genome by PCR. In our study, we found that the prevalence of HBoV1 in cancerous and non-malignant tissues was higher than that in other studies, which could be due to our particular tissue samples being compared to other stool or respiratory samples. The above-mentioned studies used different segments of the virus for detection and phylogenetic analysis, such as NS-1 and VP1/2 gene junctions, which may have influenced the results. Phylogenetic analysis of the VP1/VP2 junction by Nadji et al. showed that the isolates were HBoV-1, -2, and -3. According to the results, we determined that our isolates were HBoV-1 genotypes, so our limited positive strains could influence our findings. Karbalaie Niya et al in Iran and Abdel-Moneim et al[35] in Egypt reported that only HBoV-1 genotype was present in colorectal tumor samples without any evidence of other genotypes. Our findings were similar to their reports.

Paloniemi et al[36] surveyed the presence of HBoV in stool and nasal swab samples of pediatric patients presenting with acute gastroenteritis and/or acute RTI. They found the HBoV genome in nearly half of these cases. HBoV1 DNA was identified in 9.2 % of patients with both RTI and acute gastroenteritis but in 1.7 % of patients with gastroenteritis alone. We detected HBoV1 in about 20% of non-tumoral gastrectomy tissues, which showed mild to moderate chronic inflammation. Currently, the available data do not suggest an association between gastrointestinal symptoms and the presence of viral particles in stool samples, although a strong causal relationship will require further studies.

In our study, there was no association between age, sex, stage, and histologic subtype of the tumor and HBoV1 positivity ($P>0.05$). The rates of intestinal metaplasia and lymphoid stroma were not higher in HBoV1-positive tumors ($P>0.05$).

Limitations of our study include the lack of other complementary tests to identify HBoV, such as western blotting or IHC for viral protein expression, although we used an accurate sequencing method as a confirmatory test. We also did not perform in situ hybridization to determine where the virus was integrated. In subsequent studies, using fresh samples as well as FFPE blocks (for comparison), a larger sample size (especially in the GC group), and the use of other molecular and non-molecular-based methods are recommended to achieve more comprehensive results.

Conclusion

In conclusion, our data indicate that the presence of the HBoV1 genome is relatively high (almost 18%) in Iranian GC patients as well as in chronic gastritis tissues. Further comprehensive studies, such as in situ hybridization detection of the virus to identify the exact residing cell of the virus, detection of other genomic variants of the HBoV in the gastric tissues, and surveying the geographic distribution of the various subtypes of HBoVs are needed to gain a better understanding of the role of HBoV in carcinogenesis and related cellular damages in cancer development.

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Authors' Contribution

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Competing Interests

The authors declare no conflict of interest related to this work.

Ethical Approval

All procedures were performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the NIMAD (ethical code no: IR.NIMAD.REC.1397.337). Due to the retrospective nature and not declaring the patients' individual data in the study, the Ethics Committee was convinced that written informed consent was not necessary.

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