Received: 3 June 2023 | Revised: 15 March 2024 | Accepted: 19 March 2024

DOI: 10.1002/hsr2.2043

ORIGINAL RESEARCH

Health Science Reports Open Access WILEY

Investigation of Epstein–Barr virus, Cytomegalovirus, Human herpesvirus 6, and Polyoma viruses (JC virus, BK virus) among Gastric cancer patients: A cross sectional study

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Funding information
Tehran University of Medical Sciences and Health Services, Grant/Award Number: 58214

Abstract

Background and Aims: Gastric cancer is a significant global issue with a high death rate. This malignancy could be associated with several viral agents such as EBV, CMV, HHV-6, JCV, and BKV.

Objective: Evaluation of EBV, CMV, HHV-6 ,and JCV, BKV frequency among gastric cancer patients.

Methods: In this cross-sectional study, a total number of 60 gastric cancer specimens (32 male, 28 female) were retrieved from the pathology lab. Formalin-fixed paraffin-embedded tissue was used for molecular testing. DNA was extracted from samples, according to protocol, and used for PCR reaction. Polymerase chain reactions were used to assess CMV, EBV, HHV-6, JCV, and BKV frequency.

Results and Conclusion: The mean age of the participants was 61 years and 53.3% (32) of the participants were Male. A total number of 5 samples (8.34%) were infected with viral agents. Four male gastric samples were infected with EBV (6.67%) and only one female sample contained the BKV genome (1.67%). Totally 8.34% of

the samples were infected with EBV and BKV. The CMV, HHV-6, and JCV genome was not detected in the samples. In conclusion, the presence of two viral agents including EBV and BKV among male and female samples respectively, and the genome of other viruses were not detected.

KEYWORDS
Epstein–Barr virus, gastric cancer, infection, viruses

1 | INTRODUCTION

Gastric cancer is a significant global issue with a high mortality rate. It accounts for more than 769,000 fatalities. It is ranked fifth in terms of prevalence and fourth in terms of mortality worldwide.[1] *Helicobacter pylori* has been considered as one of the causative agents of gastric cancer development, particularly noncardia gastric adenocarcinoma.[2]

Human gastrointestinal tracts have a large proportion of viral agents. These viruses play an important role in maintaining intestinal homeostasis. However, disturbances in the virome have been linked to several diseases such as colorectal cancer, inflammatory bowel disease (Crohn's disease, ulcerative colitis), and carcinogenesis.[3,4] Disturbances in the human virome might cause intestinal inflammation and be linked.

Human herpesviruses (HHVs) are one of the frequently detected gastrointestinal tract infectious agents and might be associated with cancer development and other gastrointestinal complications.[5]

Epstein–Barr virus (EBV) is frequently widespread among the population and was observed in a small portion of gastric cancer tissues. EBV-positive gastric cancer may occur in 6.9%–8.8% of all individuals diagnosed with gastric cancer.[6]

EBV-positive gastric cancer is more prevalent in men and often found in the upper part of the stomach and multiple locations. It seems that EBV-positive gastric cancer progress is influenced by several elements including tumor suppressor genes hypermethylation, gastric mucosa inflammation, EBV's ability to evade host immune responses, and changes in cell cycle pathways.[7]

Another herpes virus that has been detected in gastric cancer is human cytomegalovirus (HCMV). It was demonstrated that increased expression of HCMV genes alters T-cell infiltration and is linked to improved survival rates in individuals with gastric cancer.[8] In tumor tissues, HCMV DNA was higher than in adjacent tissues.[9,10] In addition, GC patients were reported to have significantly higher levels of HCMV-specific IgG and IgM antibodies in their serum compared to healthy individuals.[11]

HHV-6 has been proposed as one of the causes of GI tract cancer. A bioinformatics analysis among 23 cancer types showed CMV, EBV, and HHV-6 were frequent in GI tract cancer tissues.[12] HHV-6 presence was also statistically significant in gastritis samples compared to adjacent healthy tissues.[5]

In 1971, the first two human polyomaviruses including BKV and JCV were discovered in patient samples. Both viruses cause persistent infections in the kidney, but only BKV typically causes complications at this site. On the other hand, JCV is the PML causative agent and is related to other rare neurological complications.[13]

JCV antibodies can be found in 90% of adults, except with isolated populations in South America and Papua New Guinea. JCV is typically transmitted early in life leading to a lifelong, asymptomatic infection that remains latent in the kidneys, central nervous system (CNS), and CD34+ lymphocytes.[14] IARC categorized JCV in class 2B, as a human probable biological carcinogen.[15] It has been estimated that JCV is associated with a higher risk of gastric cancer.[6] It should also be mentioned that BKV was measured in gastrointestinal cancer samples. BKV was detected in 30% (15 of 50) of the colorectal cancer samples in Pakistan.[16]

2 | OBJECTIVES

Due to the uncertainty of the importance of viral infections in gastric cancer and the necessity of establishing more robust results, in this study, we tried to measure the prevalence of CMV, EBV, HHV-6, and polyomaviruses including BKV, JCV, in the gastric cancer samples.

3 | MATERIALS AND METHODS

3.1 | Study population and sample collection

Sixty gastric cancer specimens were obtained from the pathology lab, at the Tehran University of Medical Sciences, Tehran, Iran. A microtome was utilized to create thin slices according to porotocol from tissue-paraffinized blocks, which were then transferred to 1.5 mL Eppendorf microtubes (without DNase/RNase). Before DNA extraction from paraffinized samples, deparaffinization was carried out using xylene and absolute ethanol, following previously described procedures.

3.2 | DNA extraction and quantification

Viral DNA was isolated from the samples using the phenol/chloroform method according to the instructions. The DNA quality

was assessed by a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.) and agarose gel electrophoresis. The endogenous GAPDH gene was used to confirm the quality of the extracted DNA.

3.3 | Viral genome detection

PCR was applied to detect the genome of CMV, EBV HHV-6, and BK/JC polyomaviruses. The Oligo 7.0 and NCBI was used to design the forward and reverse Primers (Table 1). The primers were constructed by an Iranian genomics company. The GAPDH gene was chosen as the housekeeping gene. PCR investigation for virus genome targeting was done according to the protocol of the Iranian master mix kit product. master mix.

3.4 | Statistical analysis

Graph Pad Prism version 8.0.2.263 was used for statistical analyses. Percentage and confidence interval were calculated according to the binomial distribution at 95% level.

4 | RESULTS

4.1 | Demographics

A total number of 60 gastric cancer samples were collected in this study, 32 (53.3%) were male and the mean age was 61 years.

4.2 | EBV, HHV-6, CMV, JCV, and BKV frequency in gastric cancer

A total number of five samples were infected with viral agents. Four male gastric samples were infected with EBV (6.67%) and only one female sample contained BKV genome (1.67%). Totally 8.34% of the samples were infected with EBV and BKV. CMV, HHV-6, and JCV genome was not detected in the samples (Table 2).

5 | DISCUSSION

Gastric cancer is generally categorized into two types based on the location of the tumor.[17] cardia cancer, which is limited to the upper part of the stomach and could be associated with gastroesophageal reflux disease (GERD).[18] Noncardia gastric cancer, is more common in the middle to lower part of the stomach and is caused by chronic inflammation of the stomach epithelium due to prolonged infection with pathogens such as EBV, HBV, and *H. pylori*.[19]

EBV-associated gastric carcinoma (EBVaGC) is a unique type of gastric cancer, accounting for 10% of all gastric malignancies.[20] A notable characteristic of this class of gastric cancer is their promoter and nonpromoter CPG island's extensive hypermethylation.[21,22]

An article conducted by Ma et al.[23] Compared the EBV positive and negative cell lines transcriptomic profiles, GC single cells and TCGA GC data sets. They exhibited that canonical WNT signaling

TABLE 2 Demographic features and types of assessed viruses among gastric cancer samples.

Variables		N (N = 60)	Percent (95% CI)[a]
Gender	Female	28	46.7% (33.7–60.1)
	Male	32	53.3% (39.9–66.4)
Mean age (years)	61		–
EBV	No	56	6.67% (1.84%–16.20%)
	Yes	4	
CMV	No	60	**0% (0.0–5.96)**
	Yes	0	
BKV	No	59	**1.67% (0.01–8.94)**
	Yes	1	
JCV	No	60	**0% (0.0–5.96)**
	Yes	0	
HHV-6	No	60	**0% (0.0–5.96)**
	Yes	0	

Abbreviations: CI, confidence interval; N, number.
[a]Was calculated based on binomial distribution.

TABLE 1 The sequences and length of primers used in this study.

Gene	Forward sequence 5′-3′	Reverse Sequence 5′-3′	Size
GAPDH	GGCCTCCAAGGAGTAAGACC	CCCCTCTTCAAGGGGTCTAC	157
HCMV	GTCAGCGTTCGTGTTTCCCA	GGGACACAACACCGTAAAGC	283
EBV	GTCATCATCATCCGGGTCTC	TTCGGGTTGGAACCTCCTTG	270
BKV	AGCAGGCAAGGGTTCTATTACTAAAT	GAAGCAACAGCAGATTCTCAACA	133
JCV	CTAAACACAGCTTGACTGAGGAATG	CATTTAATGAGAAGTGGGATGAAGAC	172
HHV-6	GCGTTTTCAGTGTGTAGTTCGGCAG	TGGCCGCATTCGTACAGATACGGAGG	521

pathway activation is common in EBV-positive GC. WNT activation was necessary for the GC invasiveness through regulating the EMT-related genes. WNT/CTNNB1 (β-catenin)/TCF7L2 axis modulated the activation of WNT in EBV+ GC and TCF7L2 expression was associated with worse outcomes and metastasis in EBV+ GCs.[23]

Another article showed that 21.4% of the 28 gastric cancer biopsies were infected with EBV. Fifty percent of EBVaGC+ individuals were men and 77.2% of EBVaGC-patients were men. The cardia had the highest rate of tumor location (17.9%) among EBVaGC+ patients.[24]

A recent systematic review exhibited 7.5% of EBV infection among 68,000 gastric adenocarcinoma samples. Among lymphoepithelioma-like gastric carcinoma, EBV prevalence was 75.9%, and 26.3% among remnant or stump carcinoma.[25]

Aversa and colleagues showed that only 0.9% frequency of gastric adenocarcinoma samples were infected with EBV in a high-incidence population in China. Aversa and colleagues found a rate of in this location.[26]

Despite the evidence that showed the participation of EBV in gastric cancer development, some studies have suggested that patients with EBV-associated gastric cancer may have a better prognosis. It is shown that EBVaGC patients exhibited a higher level of PD-L1 expression, which might make anti-PD-1/PD-L1 targeted therapy a favorable marker in EBV-associated gastric cancer patients. They reported that the pooled positivity rate for PD-L1 was 54.6% and strong association between PD-L1 and EBVaGC. Additionally, the study discovered that EBV was substantially related to gastric cancer with lymphoid stroma (GCLS), with ao detectable rate of 52.9%.[27]

Sarshari and colleagues measured the prevalence of, CMV, HHV-6 and EBV among chronic gastritis tissues and gastric cancer tumors. They found that 60% of tumor specimens and 30.9% of adjacent normal tissues had EBV, 14% and 4.7% contained CMV, and 18% and 14.2% were infected with HHV-6. EBV and CMV had a higher prevalence in the tumor tissues compared to their adjacent normal tissues. CMV prevalence was significantly higher in gastritis tissues relative to adjacent normal tissues. They also exhibited a considerable relationship between the HHV-6, CMV, and EBV viral loads with gastritis.[5] In contrast to their report, our results showed much less of EBV frequencies which is close to Hirabayashi et al.[28] We detected only 6.6% of our gastric cancer sample infected with EBV. In the measured sample we could not detect CMV and HHV-6 virus. We also found that only male patient samples contained EBV genome and none of the female samples were infected with EBV which is similar to a previous study.[28]

An interesting article conducted by Ye and colleagues showed that HCMV overexpressed US31 Gene had an association with GC patient's better overall survival and might be a good prognostic factor for GC patients. GC cells proliferation, migration, and invasion were suppressed by overexpressed US31. Expression levels of CD166, CD4, and CD66b had a positive correlation with US31 indicating the possible function of US31 to activate the immune system and regulate the tumor immune microenvironment.[29]

A meta-analysis conducted by Wang et al.[6] explored nine studies with sample size of 1356 tissue samples (692 gastric cancer and 664 control cases) and showed that the prevalence of JCV was 35.6% and it had a significant association with gastric cancer. JCV transforming antigen was detected in all of the included studies.[6]

Contrary to their findings none of the samples in our study contained JC virus genome which could be because of different reasons such as limited sample size, and environmental factors.

On the other hand, BKV genome was detected in only one of the female samples collected in our study. BK virus has been linked to some types of human cancers such as bladder cancer but the data are still inconclusive.[30] Shoraka and colleagues in a systematic review demonstrated only five included studies measured the BKV prevalence in colorectal samples and concluded that no reliable results could be interpreted from the studies.[31]

Discrepancies in our findings with other studies may stem from variations in patient genetic backgrounds, environmental factors, and sample sizes.[32] A recent meta-analysis conducted by Tavakoli and his colleagues showed that the sex difference could play role in EBV-associated gastric cancer. They found that the prevalence of EBV was higher in male patients than in female patients with gastric cancer. But interestingly they discovered that women were more likely than men to develop EBV-associated gastric cancer.[33]

Moreover, technical inconsistencies such as differences in the detection method (type and sensitivity), various sample types (fresh or fixed tissues), extraction procedures, and the potential for cross-contamination, could contribute to divergent results. For instance Casini[34] used ISH to detect BKV in colorectal cancer and detected 11 positive sample out of 18 but Sarvari and colleagues did not found any BKV in the 140 cases using PCR.[35] Interestingly Tseng and colleagues identified BKV in all of their three cases using IHC and PCR methods.[36]

6 | CONCLUSION

Different rates of viral infection have been reported among gastric cancer patients, indicating the potential role of viral agents in gastric cancer. Specifically, EBV was detected among male samples, while BKV was found among female samples.

7 | LIMITATIONS

The present study has several limitations. These include small sample sizes, a lack of classification of patients based on stomach cancer disease stages, and a lack of access to fresh tissue samples. Additionally, our results were evaluated using only one type of gastric cancer tissue specimen. Gastric tissue biopsy specimens without confirmed cancer cases were not included in this study. Furthermore, we did not conduct molecular and serological tests on patients' blood specimens to compare their status, especially systemic viremia, and its correlation with gastric tissue analysis.

AUTHOR CONTRIBUTIONS

Saber Soltani: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft; writing—review & editing. **Abbas Farahani**: Conceptualization; supervision; visualization; writing—original draft; writing—review & editing. **Ramin Shahbahrami**: Conceptualization; data curation; formal analysis; methodology; software; validation. **Zainab Shateri**: Data curation; formal analysis; investigation; methodology; resources; software; validation. **Mohammad Saeid Emadi**: Data curation; formal analysis; funding acquisition; investigation; methodology; resources; software; writing—review & editing. **Reza pakzad**: Formal analysis; investigation; methodology; software; supervision; writing—review & editing. **Maryam Lotfi**: Data curation; formal analysis; methodology; software; validation; visualization; writing—original draft. **Behzad Asanjarani**: Conceptualization; data curation; methodology; software; writing—original draft. **Arezoo Rasti**: Investigation; methodology; resources; software; visualization; writing—original draft. **Yousef Erfani**: Funding acquisition; methodology; writing—original draft. **Goli Siri**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft; writing—review & editing. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors are grateful to the Students' Scientific Research Center, Tehran University of Medical Sciences, and Tehran, Iran (58214).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be made available upon reasonable request. Saber soltani and Reza Pakzad had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

ETHICS STATEMENT

All ethical consideration were based on the Helsinki principles and reviewed by Tehran University of Medical Sciences, Tehran, Iran (ethical code: IR.TUMS.MEDICINE.REC.1401.513). All participants gave their written informed consent. This study was funded by Tehran University of medical sciences.

TRANSPARENCY STATEMENT

The lead author Goli Siri affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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How to cite this article: Soltani S, Farahani A, Shahbahrami R, et al. Investigation of Epstein–Barr virus, Cytomegalovirus, Human herpesvirus 6, and Polyoma viruses (JC virus, BK virus) among Gastric cancer patients: a cross sectional study. *Health Sci Rep*. 2024;7:e2043. doi:10.1002/hsr2.2043