Original Research Article

Characterization of Gastrointestinal Human Cytomegalovirus Infection in Biopsy Samples from Urease Positive Patients

Mojtaba Pourmomen[1], Alireza Mohebbi[2], Hanieh Bagheri[3], Alireza Nourozi[4], Alireza Tahamtan[1], Mohammad Yasaghi[2], Hamidreza Pordeli[1], *Alijan Tabarraei[3]

[1]Department of Microbiology, Islamic Azad University, Gorgan branch, Gorgan, Iran [2]Student Research Committee, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran [3]Infectious Diseases Research Centre, Golestan University of Medical Science, Gorgan, Iran [4]Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran

ABSTRACT

Introduction: Human cytomegalovirus (CMV) is a ubiquitous organism that can infect multiple organs but rarely causes gastrointestinal (GI) symptoms. However, it can be reactivated in the GI tract and lead to gastric cancer. Here, we investigated CMV infection in patients with gastric complaints. **Material and methods:** In this study, 99 biopsy samples were collected from patients with GI symptoms and CMV genome was detected by polymerase chain reaction. Sequencing was performed for computational genotyping. **Results:** We observed that 88.99% of the samples were urease positive and 5.99% of the samples were CMV positive. Moreover, genotype of the samples belonged to group 4 (gB4). There was a relatively high incidence of CMV infection in patients with GI problems. **Conclusions:** Although symptomatic CMV-associated diseases of the GI tract are rare in immunocompetent individuals, they might be considered as potential complications of CMV infection.

KEYWORDS: Human cytomegalovirus, Gastrointestinal infection, Gastric biopsy, Urease positive

***Correspondence:** Alijan Tabarraei, Address: Infectious Diseases Research Centre, Golestan University of Medical Science, Gorgan, Iran, Telephone: +98-9112733321, Email: tabarraei@goums.ac.ir

INTRODUCTION

Human cytomegalovirus (HCMV), also known as human herpes virus-5, is a ubiquitous organism from the *Herpesviridae* family [1]. HCMV infection can affect multiple organs and is a main health concern in congenitally infected infants [2, 3] and immunocompromised patients [4]. The infection is more prevalent among individuals at risk of human immunodeficiency virus (HIV) infection, drug abusers, and homosexual men infected with HIV [5]. However, HCMV-associated disease rarely develops in immunocompetent patients, and reported cases often represent mild and self-limited symptoms [6]. The virus is classified into four gB genotypes, which may influence viral invasion and pathogenesis [7]. HCMV infection often presents in two distinct forms, primary and reactivated. According to studies, the gastrointestinal (GI) tract is the most commonly involved site of HCMV infection. Young adults with HCMV infection of the GI tract may present severe and life-threatening symptoms [8]. Importantly, the risk of infection with other opportunistic gastric pathogens could increase after HCMV-induced host immune modulations. Indeed, latent HCMV infection can be reactivated following immune suppression and bacterial co-infections [9, 10], which may be related to development of de novo gastric cancer [11, 12]. Most studies have been focused on the HCMV prevalence and associated risk factors in colorectal and lower GI tract diseases, while there is limited data on the incidence of HCMV infection in the GI tract. Here, we aimed to determine the frequency of HCMV in biopsy samples of ureases positive patients with GI disorders.

MATERIALS AND METHODS

Patients

In this cross-sectional study, 99 biopsy samples were collected from patients with gastric complaints admitted to Sayyad Shirazi Hospital in Gorgan (Iran) between May 2015 and April 2016. Written consent

was obtained from all participants in the study and the study protocol was approved by the ethics committee of Golestan University of Medical Sciences (code: IR.GOUMS.REC.1396.259). The GI biopsy samples were obtained with endoscopy by a physician and were checked for the presence of *Helicobacter pylori* infection. A questionnaire was used to collect clinical and demographic data including age, gender, ethnicity and endoscopic ulcers. The samples were stored at -70 °C until analyzed.

Molecular detection of the virus

Genomic DNA was extracted from tissue samples using a commercial kit (Macherey-Nagel, Germany) and according to the manufacturer's instructions. The presence of gB region of HCMV was evaluated in all collected samples by polymerase chain reaction (PCR) using the following primers: (F) 5´-GAAACGCGCGGCAATCGG-3´ and (R) 5´-TGGAACTGGAACGTTTGGC-3´. The reaction conditions were as follows: denaturation at 95 °C for 5 min followed by 32 cycles at 95 °C for 1 min, annealing at 61 °C for 1 min, and extension at 72 °C for 1 min [13].

Genotyping of the isolates

Positive samples were sent for sequencing (Macrogen, South Korea) and the results were searched for similarity to the HCMV sequences. Genotypes of the isolated viruses were determined with computational method and using restriction enzymes RsaI and HinfI, as described previously [13, 14]. The sequences were submitted to GenBank using Bankit online submission tool with the GenBank accession numbers F435901, MF435902, MF435903, MF435904 and MF435905.

Statistical analysis

Data were analyzed with SPSS (version 16) using cross-tabulation and Pearson Chi-square tests. P-value of less than 0.05 was considered as statistically significant (95% confidence interval).

RESULTS

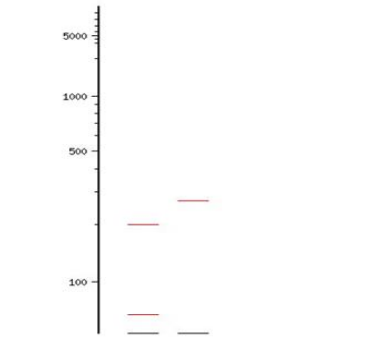
The mean age (±standard deviation) of CMV-positive and CMV-negative cases was 42.2±7.59 and 45.07±1.56 years, respectively (Table 1).

Table 1. The demographic and clinical data of HCMV-positive patients

Demographical variables (total number)		**HCMV Positive** (count/ total number)	**P-value***
Gender	Male (30)	1/30	0.696
	Female (59)	4/59	
Ethnicity	Persian (75)	5/75	0.64
	Sistani (10)	0/10	
	Turk (10)	0/10	
	Turkmen (4)	0/4	
Endoscopic Ulcer	Positive (8)	4	0.651
	Negative (53)	1	
H. pylori infection	Positive (88)	4/88	0.158
	Negative (3)	1/3	
Antibiotic use	Positive (9)	1/9	0.498
	Negative (63)	4/63	

Of all samples collected in the study, 88 (88.9%) were positive for *H. pylori* infection in the urease test. In the molecular method, the HCMV genome was detected in five (5.1%) patients. Three patients aged <45 years and two patients aged >45 years were positive. In addition, one man (3.3%) and four women (6.3%) were infected with HCMV (Table 1). Only nine patients had history of antibiotic use, one of whom was found to be CMV-positive (11.1%).

NEBcutter was used for computational digestion of the gB sequences. Accordingly, the RsaI and HinfI cutting sites on the sequenced genome were predicted (Figure 1). The results showed that all digested sequences were related to gB group 4.



#	Ends	Coordinates	Length (bp)
1	(LeftEnd)-HinfI	1-200	200
2	HinfI-HinfI	201-267	67
3	HinfI-(RightEnd)	268-303	36

#	Ends	Coordinates	Length (bp)
1	(LeftEnd)-RsaI	1-196	196
2	RsaI-(RightEnd)	241-303	63
3	RsaI-RsaI	197-240	44

Figure 1. Prediction of the electrophoretic patterns for the digestion of the sequences with HinfI and RsaI using NEBcutter. Left panel shows migration of the HinfI-treated viral DNA. Right panel right indicates electrophoretic position of the viral DNA digested with RsaI.

DISCUSSION

Colonic HCMV infection is known as the most common cause of severe GI bleeding. HCMV is usually involved in mucosal ulceration [15-21]. Pathogenic mechanisms of mucosal damage caused by HCMV and *H. pylori* underlie in their potential to stimulate α-factor over-expression, which in turn induces gastric mucosal cell proliferation and mucosal secretion [22]. In most cases, the primary HCMV infection is mild and self-limited, and rarely leads to end-stage disease. The infection in immunocompetent individuals requires no treatment. Therefore, there is a risk for opportunistic infections and HCMV-induced inflammation in non-cancerous stomach tissues [23, 24]. HCMV can act as a tumor promoter in human multi-malignant neoplasms [25]. GI diseases caused by HCMV are erosive or ulcerative and can occur from mouth down through the colorectal end. HCMV infection of the epithelial, endothelial, myocytes and fibroblast cells could result in both tissue destruction and formation of ulcers [26].

The prevalence of HCMV in gastric biopsy samples was reported to be 1% in studies conducted in India and the United States [27, 28]. In our study, the incidence of CMV infection of the GI tract was as high as 5.1%. This may require further evaluation and follow-up of the patients infected with the virus and screening for *H. pylori* infection that could reactivate latent CMV infection. Among the eight samples found positive for gastric ulcers, none was CMV positive but all were *H. pylori* positive. This indicates the possibility of *H. pylori*-induced gastric ulcers. On the other hand, three of the 53 patients with no ulcer were positive for the CMV genome, indicating the possible involvement of other factors.

Different genotypes may be involved in the pathogenesis of HCMV due to variations in genes involved in host-cell infiltration, tissue

trapping and proliferation. Based on the results of enzymatic digestion, all CMV-positive sequenced samples were identified as gB genotype 4 (Figure 1). In Brazil and USA, the most common genotypes were gB1 and gB2 [25, 26]. The HCMV gB genotypes have a significant role in pathogenicity of the virus. To our knowledge, no study has yet determined HCMV genotypes and their role in the virus pathogenesis in gastric patients. Further investigations are required to evaluate involvement of different HCMV genotypes in GI tract infection [29, 30].

CONCLUSION

Our results indicate that the prevalence of CMV infection is relatively high in patients with GI symptoms. Although symptomatic CMV-associated diseases of the GI tract are rare in immunocompetent individuals, they might be considered as a potential complication of CMV infection. Furthermore, risk factors such as infection with *H. pylori* might contribute to CMV reactivation and gastric disease progression. We have provided a supplementary material containing patients' data for future systematic review and meta-analysis.

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