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Original Article

Molecular Detection of Human Herpesvirus-8 in Plasma and Peripheral Blood Mononuclear Cells of Patients with Cryptogenic Cirrhosis

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ABSTRACT

Background: Human herpesvirus-8 is the infectious etiology of endothelial origin in tumours with blood disorders. However, in some cirrhotic patients, no etiology can be identified, and such cases are known as cryptogenic cirrhosis. The aim of this study was to determine frequencies of infection with this virus in patients with cryptogenic cirrhosis.

Methods: In the present case-control study, 67 patients with cryptogenic cirrhosis were enrolled. After the collection of plasma and peripheral blood mononuclear cell samples from the studied patients and also 70 healthy blood donors as the control group, DNA extraction was performed. All the participants were tested for viral antibodies and DNA with Enzyme Immunoassay and nested-PCR, respectively.

Results: The mean age of the studied patients was 43.8 ± 14.7 years (ranged 14–71 years), and 47 ones were male (70.1%). Out of the 67 patients, 11 ones (16.4%) were positive for antibodies, and DNA was found in plasma samples of 3 patients (4.5%), whereas the viral DNA was detected in the peripheral blood mononuclear cell samples of 5 participants (7.5%). Among 70 healthy blood donors as the control group, 3 participants (2.9%) were positive for antibodies and viral DNA was not detected in plasma and peripheral blood mononuclear cell samples.

Conclusion: Based on the results of this study, the prevalence of infection in patients with cryptogenic cirrhosis is higher than that in the general population. According to these results, it seems that infection with this virus should be considered in patients with cryptogenic cirrhosis. However, more evidence is needed to prove this.

Keywords: Human Herpesvirus 8, Liver Cirrhosis, Nested PCR

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Introduction

Cryptogenic cirrhosis, determined as an end-stage of a chronic liver disease, has an etiology remained unclear after performing laboratory, clinical and pathological assessments. To prove the presence of cryptogenic cirrhosis, a number of possible causative etiologies, such as viral and autoimmune hepatitis, alcohol abuse, non-alcoholic steatohepatitis (NASH), hepatotoxic drug, any severe systemic disease, biliary tract disease, decompensated diabetes, Wilson's disease, haemochromatosis, thyroid dysfunction and so on, must be excluded (1, 2). The prevalence rate of cryptogenic cirrhosis was about 5% to 30% in individuals with cirrhosis in earlier studies (3), but it has been decreased to around 5% with progress in this field and with the advancement of viral hepatitis diagnosis and other biomarkers (2, 4). Cryptogenic cirrhosis is the fourth reason for liver transplantation all over the world, and nearly 7%-14% of the transplant recipients receive these transplants due to this etiology (2, 3).

Liver cirrhosis with high mortality is developing in the world, whereas cryptogenic cirrhosis is a chronic liver impairment for which it is not possible to identify an etiologic factor by laboratory, clinical and histological findings. Recently, for some patients with cryptogenic cirrhosis, several viruses are being considered as the possible etiologic factors, but the association between the viral infections and cryptogenic cirrhosis is still remaining unclear (5).

Human herpesvirus-8 (HHV-8) is one of the oncogenic human viruses which is a member of the family *Herpesviridae* and belongs to the subfamily *Gammaherpesvirinae*. Kaposi's sarcoma-associated herpesvirus (KSHV) is an informal name for HHV-8. This virus can cause Kaposi's sarcoma, which is a type of cancer that commonly occurs in individuals suffering from acquired immune deficiency syndrome (AIDS) (1, 6), primary effusion lymphoma (7) and also several types of multi-centric Castleman's disease. Thereby, the virus is one of the known viral causes of human cancers (8).

In total, worldwide distribution of HHV-8 is low to moderate in Western and Asian general populations, while it is higher in African populations. The HHV-8 transmission routes may also differ in distinct geographic areas, sexual and nonsexual and blood-borne transmission (9).

Previous studies showed that the prevalence and routes of HHV-8 infection vary substantially among different social groups in Iran. The prevalence of HHV-8 in the general population of Iran is about 4%. However, the human immunodeficiency virus (HIV) infected patients show a significant higher prevalence (about 8%) and the infection rate has peaked (13.3%) among intravenous drug users with high-risk sexual behaviors (10).

Human herpesvirus-8 among patients with advanced HIV infection or people with cirrhosis is frequently known as an oncogenic factor resulting in hepatocellular carcinoma (HCC); however, the prevalence of HHV-8 infection is uncertain (8,11, 12). The pathogenic role of some oncogenic viruses, like HHV-8, in hepatitis diseases and their complications, especially cryptogenic cirrhosis, are factors which require more investigations (13-16). The HHV-8 represents high variability in genome. This variability is remarkable in 3´ and 5´ of genome. There are three overlapping genotyping methods for HHV-8 based on the K1, K15 and ORF26 (genotypes are A, A5, B, C, D, E, F, Z, P, M, B, Q, R and N) (17). Due to the current understanding of the HHV-8 importance and pathogenesis, there are some novel therapeutic approaches and vaccines are developing for this particular virus (17). Human herpesvirus-8 DNA is reported to be found in some types of liver diseases which are prevalent in HCC patients, but in cirrhotic patients, the liver is mostly destroyed by multiple causes of cirrhosis (18, 19).

It has been reported that the seroprevalence of HHV-8 in individuals with moderate to severe liver cirrhosis was significantly higher than that in healthy people (20-22). It appeared to have high correlation with sex, cirrhosis severity, hepatitis B virus, disease severity, alcoholism, and thrombocytopenia (20). By considering the current developing therapies and vaccines for HHV-8, the diagnosis and finding a clear relation between the HHV-8 infection and cirrhosis could be promising. The present study aimed to assess the frequency of HHV-8 infection in plasma and peripheral blood mononuclear cell (PBMC) samples of Iranian patients with cryptogenic cirrhosis.

Materials and Methods

Patients and inclusion criteria

Participants with Probable diagnosis of liver cirrhosis were eligible for inclusion in this case

control-study. Sixty-seven patients with affirmative cryptogenic cirrhosis referred to hospitals affiliated to Iran University of Medical Sciences (IUMS), Tehran, Iran from November 2015 to February 2017 were enrolled in this research. Based on previous studies (1, 23, 24), inclusion criterion was considered as having laboratory tests showing abnormal liver function with unknown etiology. The diagnosis of cryptogenic cirrhosis was made after a comprehensive screening as follows:

(1) The presence of abnormal liver function tests with unknown etiology for over 6 months prior to this research (have been tested every 3 months)

(2) All of the participants had anti-HCV antibodies and were plasma HCV-RNA negative (have been tested for more than two times before entry to this research)

(3) Exclusion of all other known etiologies of liver disease based on laboratory, clinical, and epidemiological information, such as HBV infection (HBV-DNA and hepatitis B surface antigen [HBsAg] negative), genetic disorders, alcohol intake, drug toxicity, and autoimmunity (negativity for anti-mitochondrial, antinuclear antibodies, etc.)

(4) Being negative for HIV infection (anti-HIV antibodies negative) (23).

Liver cirrhosis was confirmed by a pathological method using liver biopsy specimen in some of the patients, and the rest of the patients were diagnosed by transient elastography (TE). This technique (TE) is suitable for recognition of the patients with various grades of fibrosis and also has a great ability to exclude the stage of hepatic cirrhosis. On the other hand, it is highly reproducible and reliable, easy to use, and rapid (24,25).

Blood samples and PBMC isolation

About 5mL of peripheral blood was taken from all studied patients and seventy healthy controls into sterile EDTA-containing vacutainer tubes. After the separation of plasma from whole blood, PBMCs were isolated from the blood by a standard procedure of Ficoll–Hypaque gradient centrifugation (Lympholyte H, Cedarlane, and Hornby, Canada). The isolated PBMCs were washed 3 times with phosphate-buffered saline (PH=7.3±0.1) and frozen at –80°C for DNA extraction.

HHV-8 serological assessment

Anti-HHV8 antibodies were determined in plasma samples using enzyme-linked immunosorbent assay (ELISA) kit (Advanced Biotechnologies Inc., Eldersburg, MD, USA), according to the manufacturer's instructions.

HHV-8 molecular detection

Diagnosis was made based on standard molecular examination and the HHV-8 DNA in plasma specimens was extracted using NucleoSpin® Dx Virus kit (Macherey-Nagel, Duren, Germany), according to the manufacturer's instructions. Viral DNA was extracted from a pellet of PBMC sample (about $4–6 \times 10^6$ cells) by the QIAamp® DNA Mini Kit (Qiagen GmBH, Hilden, Germany) with a protocol adapted from the manufacturer's instructions.

Detection of HHV-8 genomic DNA in plasma and PBMC specimens were carried out by nested-PCR method. Briefly, a set of nested primers from the region of ORF-26 (virus minor capsid) of herpesvirus-like sequences in KS had been described (6, 12, 26-29) and used in our previous study. Details of primers are listed in Table 1.

Table 1. Primers for Human Herpesvirus-8 ORF26 coding region

Application	Direction	Name	Sequences
The first step of PCR	Sense	KSA	5´-AGC CGA AAG GAT TCC ACC AT-3´
	Antisense	KSB	5´-TCC GTG TTG TCT ACG TCC AG-3´
The second step of PCR	Sense	KS1	5´-TAT TCT GCA GCA GCT GTT GG-3´
	Antisense	KS2	5´-TCT ACG TCC AGA CGA TAT GTG C-3´

The first round of the PCR was performed in a 25 µl mixture consisting of the extracted sample, *Taq* DNA polymerase, PCR buffer with $MgCl_2$, dNTPs, and 10 pM of each outer primer. In the second PCR amplification for nested PCR, the same procedure and with the inner primer pairs and PCR product from the first step was used. Amplification performed for both steps as follows initial denaturing for 5 min at 95°C, and 38 cycles of 95°C for 40 sec, 55°C for 40 s, and 72°C for 37 s, followed by a final extension at 72°C for 3 min. PCR products (138-bp) with negative and positive controls and DNA size markers (ladder 100-bp) were visualized by 1.5% agarose gel electrophoresis with SYBR Green staining (28, 30). For ensuring the accuracy of gene amplification, PCR test was performed for β-globin gene amplification on each, using primers PCO4 and a CH20 marker (31, 32).

Statistical evaluation

All data were entered into the SPSS version 20 (SPSS Inc., Chicago, IL, USA). Continuous data were recorded as means ± SD or medians, and categorical data were analysed using chi-square tests. Statistical significance was set at $p<0.05$. A Fisher's test was used to assess the significance between gender and HHV-8.

Results

Demographic data

Sixty-seven patients with established cryptogenic cirrhosis and also 70 people as healthy control group were enrolled in the present study. The results of HCV RNA and HBV DNA detection, and also of anti-HCV antibodies, HBsAg, HBcAb, HBeAg, and HBeAb, were repeatedly negative. The mean age of studied patients was 43.8 ± 14.7 years (ranged 14-71 years), and 47 patients (70.2%) were male.

HHV-8 detection

Out of the 67 patients with cryptogenic cirrhosis, 11 ones (16.4%) were positive for anti-HHV8 antibodies and HHV8-DNA was found in plasma samples of 3 patients (4.5%), whereas the HHV8-DNA was detected in the PBMC samples of 5 ones (7.5%).

Among 70 healthy blood donors as control group, 3 participants (2.9%) were positive for anti-HHV8 antibodies and HHV8-DNA was not detected in plasma and PBMC samples. Furthermore, more data about the HHV-8 status and other clinico-pathologic data are summarized in Table 2. Compared with patients with HHV-8 positive, patients with HHV-8 negative represents statistically significant older age (44 ±16.1 years and 41 ±14.0 years respectively, $p = 0.71$). Furthermore, patients with HHV-8 positive were only males [3 (6.4%), $p = 0.54$]. Variables were explored for their association with HHV-8 at SPSS20 and were considered for inclusion in the regression model.

The relationship of the HHV-8 DNA status with the cryptogenic cirrhosis clinical parameters are represented in Table 2. HHV-8 positivity was not related to gender, age, history of surgery, blood transfusion, endoscopy, jaundice and AFP levels ($p>0.05$). A significant association was observed between the presence of HHV8-Ab and the detection of HHV8 DNA in plasma specimen ($P = 0.003$) and HHV8 DNA in PBMC samples ($P < 0.001$). In addition, the level of haemoglobin was significantly differed between the two serological groups ($P = 0.031$). Demographic information, laboratory and epidemiological parameters of cryptogenic cirrhotic patients and comparison between patients with positive and negative results for HHV-8 infection are summarised in Table 2. The comparison of patients with cryptogenic cirrhosis and healthy people in terms of demographic characteristics, epidemiological, and laboratory parameters is shown in Table 3. There is a statistically significant difference between Cryptogenic Cirrhosis and healthy groups in HHV-8 antibody ($P=0.024$).

Table 2. Demographic Characteristics, Epidemiological, and Laboratory Parameters in the studied patients with Cryptogenic Cirrhosis with and without HHV8-Ab

Parameters		HHV8-Ab Positive	HHV8-Ab Negative	Total	*P*. value
No. of patients		11 (16.4%)	56 (83.6%)	67 (100%)	0.930 T test
Gender Male/Female		9.2	38.18	47.20	0.484 Fisher's Exact Test
Age (Year) ± SD		42.5 ± 12.5 (24-57)	44.3 ± 14.3 (19-71)	43.9 ± 14.0 (19-71)	0.697 T test
Laboratory Parameters:					
HHV8 DNA in Plasma		3 (27.3%)	0 (0.0%)	3 (4.5%)	0.003[d] Fisher's Exact Test
HHV8 DNA in PBMC[a]		5 (45.5%)	0 (0.0%)	5 (7.5%)	<0.001[d] Fisher's Exact Test
White Blood Cell		4147 ± 2439 (1800-8700)	4322 ± 1777 (1200-9600)	4294 ± 1881 (1200-9600)	0.780 T test
Hemoglobin (gr/dl)		11.6 ± 2.0 (8.4-14.6)	12.9 ± 1.8 (8.3-15.7)	12.7 ± 1.9 (8.3-15.7)	0.031[d] T test
ALT[b] (IU/L)		57.1 ± 64.0 (18-244)	62.3 ± 65.4 (10-378)	61.5 ± 64.8 (10-378)	0.431 Mann-Whitney U Test
AST[c] (IU/L)		78.1 ± 100.2 (22-376)	76.2 ± 75.3 (19-400)	76.5 ± 79.0 (19-400)	0.271 Mann-Whitney U Test
Bilirubin Total (mg/dl)		1.76 ± 1.2 (0.6-4.2)	2.6 ± 2.5 (0.6-17.2)	2.5 ± 2.4 (0.6-17.2)	0.119 Mann-Whitney U Test
Cholesterol (mg/dl)		180.7 ± 52.6 (107-270)	157.9 ± 33.8 (68-240)	161.7 ± 38.0 (68-270)	0.069 T test
Triglyceride (mg/dl)		132.2 ± 54.3 (61-211)	116.4 ± 50.0 (30-346)	118.9 ± 50.6 (30-346)	0.348 T test
Epidemiological parameters:					
History of surgery		9 (81.8%)	37 (66.1%)	46 (68.7%)	0.481 Fisher's Exact Test
History of blood transfusion		5 (45.5%)	26 (46.4%)	31 (46.3%)	1.000 Fisher's Exact Test
History of endoscopy		11 (100.0%)	53 (94.6%)	64 (95.5%)	1.000 Fisher's Exact Test
History of jaundice		1 (9.1%)	8 (14.3%)	9 (13.4%)	1.000 Fisher's Exact Test
Body-Mass-Index (BMI) kg/m^2		23.3 ± 4.8 (17.4-34.6)	24.6 ± 5.0 (16.9-39.1)	24.4 ± 5.0 (16.9-39.1)	0.437 T test
Education	**Under diploma**	7 (63.6%)	31 (55.4%)	38 (56.7%)	0.745 Chi-Square
	Upper diploma	4 (36.4%)	25 (44.6%)	29 (43.3%)	
Marital Status	**Single**	5 (45.5%)	13 (23.2%)	18 (26.9%)	0.149 Chi-Square
	Married	6 (54.5%)	43 (76.8%)	4973.1%)	

a: Peripheral Blood Mononuclear cells, b: Alanine aminotransferase (ALT), c: Aspartate aminotransferase (AST), d: Statistically Significant

Table 3. Demographic Characteristics, Epidemiological, and Laboratory Parameters in the studied patients with Cryptogenic Cirrhosis and Healthy Controls

Parameters		Healthy Controls	Patients with Cryptogenic Cirrhosis	*P.* value
No. of patients		70	67	
Gender Male/Female		51.19	47.20	0.850 Fisher's Exact Test
Age (Year) ± SD		41.7 ± 13.2 (18-70)	43.9 ± 14.0 (19-71)	0.238 T test
Laboratory Parameters:				
HHV8 Abs		3 (4.3%)	11 (16.4%)	0.024[d] Fisher's Exact Test
HHV8 DNA in Plasma		0 (0.0%)	3 (4.5%)	0.114 Fisher's Exact Test
HHV8 DNA in PBMC[a]		0 (0.0%)	5 (7.5%)	0.026[d] Fisher's Exact Test
White Blood Cell		6319 ± 1409 (4077-9400)	4294 ± 1881 (1200-9600)	<0.001[a] T test
Hemoglobin (gr/dl)		13.0± 2.1 (10-18)	12.7 ± 1.9 (8.3-15.7)	0.366 T test
ALT[b] (IU/L)		21.03 ± 8.1 (5-39)	61.5 ± 64.8 (10-378)	<0.001[d] Mann-Whitney U Test
AST[c] (IU/L)		23.4 ± 8.2 (6-41)	76.5 ± 79.0 (19-400)	<0.001[d] Mann-Whitney U Test
Bilirubin Total (mg/dl)		0.8 ± 0.3 (0.3-1.6)	2.5 ± 2.4 (0.6-17.2)	<0.001[d] Mann-Whitney U Test
Cholesterol (mg/dl)		139.2 ± 46.2 (59-253)	161.7 ± 38.0 (68-270)	0.002 T test
Triglyceride (mg/dl)		158.5 ± 43.8 (98-269)	118.9 ± 50.6 (30-346)	<0.001[d] T test
Epidemiological parameters:				
History of surgery		5 (7.1%)	46 (68.7%)	<0.001[d] Fisher's Exact Test
History of blood transfusion		2 (2.9%)	31 (46.3%)	<0.001[d] Fisher's Exact Test
History of endoscopy		1 (1.4%)	64 (95.5%)	<0.001[d] Fisher's Exact Test
History of jaundice		2 (2.9%)	9 (13.4%)	0.023[d] Fisher's Exact Test
Body-Mass-Index (BMI) kg/m^2		24.0 ± 6.2 (17-39)	24.4 ± 5.0 (17-39)	0.798 T test
Education	**Under diploma**	40 (57.1%)	38 (56.7%)	1.000 Chi-Square
	Upper diploma	30 (42.9%)	29 (43.3%)	
Marital Status	**Single**	18 (25.7%)	18 (26.9%)	1.000 Chi-Square
	Married	52 (74.3%)	49 (73.1%)	

a: Peripheral Blood Mononuclear cells, b: Alanine aminotransferase (ALT), c: Aspartate aminotransferase (AST), d: Statistically Significant

Complete information about HHV8 Ab positive patients with cryptogenic cirrhosis has been summarized in Table 4. The age ranged from 24 to 57 years in HHV8 Ab positive patients with cryptogenic cirrhosis. Also, great range of difference in liver enzymes were represented in these patients.

Table 4. Demographic information of HHV8 Antibody Positive Patients with Cryptogenic Cirrhosis and Healthy Blood Donors

Number of the patients	Gender/Age	Education	Marital status	ALT[c] (IU/L)	AST[d] (IU/L)	HHV8 Abs
P-3	24/M[a]	Under diploma	Single	244	376	+
P-6	43/M	Upper diploma	Married	35	56	+
P-10	40/M	Under diploma	Single	44	46	+
P-15	57/M	Upper diploma	Married	50	57	+
P-26	53/F[b]	Upper diploma	Single	76	82	+
P-31	30/F	Under diploma	Single	42	67	+
P-42	40/M	Under diploma	Married	22	30	+
P-53	56/M	Under diploma	Married	42	37	+
P-58	57/M	Under diploma	Married	31	46	+
P-64	24/M	Under diploma	Single	18	22	+
P-66	43/M	Upper diploma	Married	24	42	+
H-70	22/M	Under diploma	Single	29	32	+
H-114	44/M	Upper diploma	Married	19	20	+
H-133	31/M	Upper diploma	Single	23	24	+

a: Male, b: Female, c: Alanine aminotransferase (ALT), d: Aspartate aminotransferase (AST)

Discussion

To the best of our knowledge, this is the first research to determine the frequency of HHV-8 infection in plasma and PBMC specimens of patients with cryptogenic cirrhosis in Iran; therefore, there are no similar studies for comparative purposes. Thus, the results of the present study can be compared with the reports obtained from the people at risk for HHV-8 infection for instance, the HHV-8 condition in people with HIV infection, intravenous drug users (IDUs), patients with liver cirrhosis and individuals with chronic hepatitis (10, 33).

It has been reported that the frequency of HHV-8 DNA is 13.3% in IDUs (especially those who had high-risk sexual behaviours), 8% in HIV-infected patients, and 3.6% in general population in Iran (10). Also, patients with liver cirrhosis have a higher prevalence of HHV-8 infection in comparison with healthy controls, especially those with hepatitis B virus (HBV) infection, severe cirrhosis, and alcoholism (21). On the other hand, it has been shown that old age and hepatitis are two risk factors for infection with HHV-8 in patients with liver cirrhosis (34). It has been determined that high HHV-8 seroprevalence is present in patients with chronic hepatitis prior to the development of cirrhosis, especially in patients with hepatitis C. In addition, old age seems to play an important role in HHV-8 seroprevalence in patients with chronic hepatitis (33).

In patients with cryptogenic cirrhosis in the immunocompromised status, the presence of HHV-8 DNA may be a consequence of reactivation of the latent virus (1,7, 24). Although, presumably, the frequency of HHV-8 infection is not related to the etiology of cirrhosis in patients with liver cirrhosis, in clinical forms of hepatic cirrhosis, this infection seems to be more frequent (14, 35, 36).

In the present study, it was found that 16.4% of the studied patients were positive for anti-HHV8 antibodies, and HHV-8 DNA was detected in plasma samples of 3 patients (4.5%) and also in PBMC specimens of 5 participants (7.5%). As shown in Table 2, no significant association was observed between the presence of HHV8 DNA in plasma specimens and gender ($P = 0.54$) of the participants. There are reports which show that HHV8 DNA is detected mainly in elderly participants, and an age-related increased risk of classic Kaposi's sarcoma has been found among Asian and African people (37, 38). Also, Tseng and colleagues showed that HHV-8-seropositive cirrhotic patients were significantly older than seronegative studied patients (34). It seems that these results are contradictory with the results of the present study and there is a need for further studies in this regard. Even though, these differences could be justified based on the studies population and laboratory diagnostic methods.

On the other hand, in the current study, HHV-8 seropositivity in patients with cryptogenic cirrhosis (16.4%) was higher than that in healthy controls (2.9%). Therefore, it seems that researchers should investigate the possibility of the effect of this infection on the development of cryptogenic liver cirrhosis. Meanwhile, these results need to be confirmed by further studies in this field.

In a retrospective cohort study by Wootton *et al*. (38) where clinical outcomes of cirrhosis have been compared in patients with non-cryptogenic cirrhosis, it is reported that cryptogenic cirrhosis is associated with metabolic syndrome. HHV-8 DNA is occasionally found in some types of liver disease. Transmission of HHV-8 depends on some risk factors, including injecting drugs and unsafe sexual behaviours (19,34,39,40). However, there was no agreement in the association of gender with the prevalence of serum HHV-8 DNA. Today, cryptogenic cirrhosis remains a common clinical condition although recent advances have allowed for a better knowledge of underlying causes (41, 42). We found an absolute proportion of males in patients with HHV-8 compared with negative cases. This is somewhat not significant, but may be explained by the greater proportion of high-risk behaviours in the male group, which are characteristically male diseases in the world.

There are several limitations to this study. The lack of difference in related parameters for cirrhosis may be due to the smaller number of patients in this category. However, the authors were not able to clarify the HHV-8 association with cryptogenic cirrhosis. Indeed, HHV-8 could have some cytolysis degradation but the possible role of this herpesvirus in cirrhosis needs further studies. In addition, there are limited data in this particular field of study, especially in recent years. This limitation reflects in comparing the results of the current study with other relevant studies and reaching a clear conclusion.

As noted earlier, the number of cryptogenic cirrhosis cases decreases each year due to the identification of the causes of liver cirrhosis. For example, Keyvani *et al.*(23) reported that 8.9% of the Iranian patients with cryptogenic cirrhosis who were liver transplantation candidates had occult HCV infection (OCI) and also, Hashemi *et al*. (43) reported that 14% of the Iranian patients with cryptogenic cirrhosis had occult HBV infection (OBI)). Therefore, it seems that a prospective study should be designed to investigate the various microbial agents causing cryptogenic cirrhosis.

Molecular detection of HHV8 DNA in plasma and PBMC specimens, as two major blood compartments, showed lytic and latent infections, respectively (12). In the present study, HHV8-DNA was detected in plasma specimens of 3 (4.5%) and in PBMC samples of 5 (7.5%) participants with cryptogenic cirrhosis. Therefore, three patients out of five (60.0%) were positive for HHV8 DNA in their plasma sample. It should be noted that detection of HHV8 DNA in plasma specimens is expected, mostly during viremia phases.

This condition has already been reported in patients with HIV infection by Hesamizadeh *et al*. They were detected HHV8 DNA in PBMC and plasma samples of six (5.5%) and four (3.6%) HIV infected patients, respectively (12). The current study is the first research to determine the frequency of HHV8 infection in Iranian patients with cryptogenic cirrhosis; therefore, there are no similar reports available for comparative purposes.

Conclusion

In conclusion, this study indicated that in the patients with cryptogenic cirrhosis, more than 7% suffered from HHV-8 infection; therefore, it seems that this infection should be considered in these patients. The diagnosis of HHV-8 infection is not a routine procedure in Iran. According to the data of this study, the prevalence of this infection among patients with cryptogenic cirrhosis is relatively high, and thus the screening of HHV-8 infection may be beneficial in these patients. It seems that further studies will be helpful to define risk factors, epidemiology, and also related malignancies associated with HHV8 infection in various high-risk groups in Iran.

Acknowledgment

This study was approved by the local ethics committee of Iran University of Medical Sciences and the informed consent forms from all patients were obtained.

Authors' contributions

Farah Bokharaei-Salim and Maryam Esghaei designed the present study and were responsible for the overall management of the study; Khashayar Hesamizadeh organized the analysis of the study; Mohammad Hossein Razizadeh and Hossein Keyvani prepared the manuscript. The statistical analyses were conducted by Fatemeh Haghparvar. All authors contributed to the final version of the manuscript.

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Conflict of interest

The authors declare no conflict(s) of interest.

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