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ORIGINAL ARTICLE

Investigation of Hepatitis E Seroprevalence in HIV Positive Patients by a Novel Enzyme Linked Fluorescent Assay Test

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SUMMARY

Background: **Hepatitis E virus (HEV) infection is usually an acute self-limiting disease, which causes rapidly progressive cirrhosis and chronic infection in patients with hematological malignancies, patients requiring chemotherapy, and HIV-infected patients. The aim of this study was to investigate the positivity of hepatitis E IgM and IgG in HIV positive patients with the recently introduced Enzyme Linked Fluorescent Assay (ELFA) commercial kits.**

Materials and Methods: **The study included 126 patients who were followed up by the Infectious Diseases and Clinical Microbiology Clinic of Sakarya University Training and Research Hospital between October 2017 and December 2018 for HIV positivity. Serum samples of the patients were evaluated for anti-HEV IgG and IgM positivity with a novel commercially available kit using the ELFA method (bioMerieux, France). The study group consisted of 126 patients with HIV infection. Anti-HEV IgG antibodies were studied primarily from plasma samples. Anti-HEV IgM positivity was also investigated in patients with anti-HEV IgG positivity.**

Results: **The study group consisted of 114 (90.5%) males and 12 (9.5%) females with a mean age of 38.11 ± 13.32 (min: 18, max: 80) years. Anti-HEV IgG was positive in 5 (4.0%) HIV-positive patients. One of the anti-HEV IgG positive patients was newly diagnosed with HIV and the other four patients were being followed up for HIV positivity. Anti-HEV IgM was negative in all patients. None of the patients with anti-HEV IgG positivity had anti-HCV and HBsAg positivity.**

Conclusions: **In the study, anti-HEV IgG positivity was found to be 4% in HIV-positive individuals, and no HCV and HBV co-infection was detected in any patients with HIV and HEV coexistence.**

HEV infections do not emerge as a priority among HIV-infected people, but HEV should also be investigated in HIV-infected individuals with liver abnormalities of uncertain etiology.

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INTRODUCTION

Hepatitis E virus (HEV) is a hepatitis agent spread by fecal-oral transmission via water contaminated with feces [1]. Hepatitis E virus (HEV) is a non-A and non-B hepatitis agent transmitted by fecal-oral route and possibly spread with feces [1]. HEV, which was previously placed in the Caliciviridae family, was then classified into the Hepeviridae family as Orthohepevirus. Within

this genus, genotypes 1 - 4 and 7 have been associated with human infections [2]. After approximately 2 to 8 weeks of incubation, HEV infection occurs with the clinical findings that are similar to other acute viral hepatitis infections [3]. It is usually a self-limiting disease. It is asymptomatic especially in children [4]. However, it may result in fulminant liver failure in pregnant women and those with chronic liver disease. Although it has been thought that hepatitis E infections have not become chronic until recently, it may cause chronic HEV infections in organ transplant recipient patients, and immunosuppressive therapy and HIV-infected patients [5]. The characteristics of infections and clinical severity of diseases may vary according to the genotype. Underdeveloped countries, especially in Asia and Africa, are highly endemic regions, and genotypes 1 and 2 are often responsible for infections [2]. The transmission of these genotypes occurs by fecal-oral route and occurs as major water-borne outbreaks or sporadic infections. However, in developed countries, HEV transmission is of zoonotic origin occuring as sporadic or autochthonous cases, and genotypes 3 and 4 are often detected [5]. In the high-endemic countries such as China and India, HEV seroprevalence is reported to be over 25% in the general population, while it is reported to be around 2% in Europe and about 3% in America [3]. In our country, a study was published in 1993 and covered five different regions of Turkey. It has been reported that the total HEV seropositivity rate was 5.9% (80/1,350) [6]. The seroprevalence of HEV varies widely in various regions of our country in different study groups and varies between 0 - 73% [7].

There are studies that report HEV antibodies are more common in HIV patients, as well as studies that have a similar prevalence of HEV antibodies in HIV-infected and non-HIV-infected individuals. We could not find a comprehensive study in this field in our country. In this study, we investigated the seroprevalence of HEV in HIV-positive patients with a novel commercial kit that uses the ELFA method.

MATERIALS AND METHODS

This study retrospectively evaluated the plasma samples of patients who were admitted to the Clinical Microbiology Laboratory of Sakarya University Education and Research Hospital between October 2017 and December 2018 for HIV infection.

The study group consisted of 126 patients with positive HIV-1 viral load. The Ethics Committee of Sakarya University Faculty of Medicine approved the study with the number 71522473/050.01.04/289 dated 12.12.2018. The samples of the patients who were sent to Sakarya University Education and Research Hospital Clinical Microbiology Laboratory between October 2017 and December 2018 for diagnosis and follow-up due to HIV infection are taken into study and HIV-1 RNA viral load was studied without delay from the plasma samples of the patients on a GeneXpert device (Cepheid, USA). This system yields quantitation in the range of 40 - 10,000,000 copies/mL, yielding rapid results and subgroups of HIV-1 M Group A, B, C, D, AE, F, G, H, AB, AG, J, K, N, and O. It is an *in vitro* system which can detect subtypes of groups over 95% sensitivity and specificity. All samples detected with HIV-1 RNA viral load positivity with this system were stored in a deep freezer at -80°C until the enzyme-linked fluorescent assay (ELFA) run time. Anti-HEV IgG antibody tests (bioMerieux, France) have been studied in accordance with the manufacturer's instructions. It is intended to aid in the diagnosis of hepatitis E infection in patients with hepatitis symptoms and/or clinical symptoms. Anti-HEV IgM was investigated and a 100 µL plasma sample was used for the ELFA method as suggested. Threshold concentrations for anti-HEV IgG and anti-HEV IgM tests were 0.56 U/mL, whereas values below < 0.56 U/mL were interpreted as negative, values above ≥ 0.56 U/mL were considered positive. The sensitivity of the test was reported to be between 59.15% and 97.65% in the immunocompetent and immunosuppressed patient groups when compared with molecular methods in the viremic and post-viremic periods.

The retrospective records of the patients were screened for simultaneous ALT (0 - 50 U/L), AST (0 - 50 U/L), CD4 (mm^3), CD8 (mm^3), total lymphocyte (mm^3), HIV-1 RNA viral load (copy/mL), anti-HCV, HBsAg, anti-HAV IgM, anti-HAV IgG, VDRL levels and were evaluated.

Statistical analysis

The data were evaluated with SPSS for Windows V 20.0 program. Chi-square and Fisher's exact tests were used for comparisons between the groups. The *t*-test and Mann Whitney *U* test according to normality were used to compare the means. $p < 0.05$ was considered statistically significant.

RESULTS

In this study, 114 (90.5%) members of the study group were male and 12 (9.5%) were female. The mean age was 38.11 ± 13.32 (min: 18, max: 80) years.

Anti-HEV IgG was positive in 5 (4.0%) HIV-positive patients. All patients with anti-HEV IgG positivity were male and the mean age was 51.8 ± 10.5, which was significantly higher than the other patients ($p = 0.023$), (Table 1).

One of the anti-HEV IgG positive patients was newly diagnosed with HIV and the other four patients were being followed up for HIV positivity. Anti-HEV IgM was not positive in any patient. Some features of patients with anti-HEV IgG positive are given in Table 2.

When we evaluated the CD4 counts of the patients, 24 (19.0%) patients had CD4 levels below 200 cells/µL. However, none of these patients had anti-HEV IgG positivity. No statistically significant difference was found

Table 1. Distribution of some variables according to anti-HEV IgG positivity.

	Anti-HEV IgG positive n = 5	Anti-HEV IgG negative n = 121	p
Age, mean ± SD*	**51.8 ± 10.5**	**37.5 ± 13.1**	**0.018**
Gender n (%)			
Man	**5 (4.4)**	**109 (95.6)**	**0.601**
Woman	**0 (0)**	**12 (100)**	
CD4 (cell/µL)			
< 200	**0 (0)**	**24 (100)**	
200 - 499	**2 (4.0)**	**48 (96.0)**	**0.488**
500 +	**3 (5.8)**	**49 (94.2)**	
HBsAg			
Positive	**0 (0)**	**8 (100)**	**0.716**
Negative	**5 (4.2)**	**113 (95.8)**	
Anti HCV			
Positive	**0 (0)**	**2 (100)**	**0.922**
Negative	**5 (4.0)**	**119 (96.0)**	
HAV IgG			
Positive	**2 (2.6)**	**75 (97.4)**	**0.376**
Negative	**3 (6.1)**	**46 (93.9)**	
HAV IgM			
Positive	**-**	**-**	**-**
Negative	**5 (4.0)**	**121 (96.0)**	
VDRL			
Positive	**0 (0)**	**18 (100)**	**0.456**
Negative	**5 (4.6)**	**103 (95.4)**	

SD - standard deviation.

Table 2. Characteristics of patients with anti-HEV IgG positive.

Patient number	Gender / age	Diagnosis	Viral load (copy/mL)	ALT (U/L)	AST (U/L)	Cd4 %	Cd8 %	Lymphocytes
1	**M/48**	**old**	**2,030**	**27**	**23**	**14.1**	**62.0**	**3,920**
2	**M/61**	**old**	**3,280**	**25**	**30**	**26.7**	**51.8**	**1,570**
3	**M/35**	**old**	**< 40**	**42**	**25**	**40.6**	**41.3**	**1,850**
4	**M/58**	**old**	**< 40**	**11**	**13**	**22.5**	**59.2**	**2,280**
5	**M/57**	**old**	**60,000**	**16**	**27**	**23.8**	**49.5**	**1,430**

M - Male.

between CD4 count and anti HEV IgG positivity (p = 0.488), (Table 1).

None of the patients with anti-HEV IgG positivity had anti-HCV and HBsAg positivity. Anti-HAV IgG was positive in 77 (61.1%) patients and anti-HAV IgM was negative in all patients. Anti-HCV positivity was detected in 2 (1.6%) patients, HBsAg positivity in 8 (6.3%) patients, and VDRL positivity in 18 (14.3%) patients (Table 1).

There was no statistically significant difference in ALT, AST, CD4, and CD8, and HIV RNA viral load values in anti-HEV IgG positive individuals (p > 0.05 for each).

Table 3. Distribution of some biochemical values of patients according to anti-HEV IgG status.

	Anti-HEV IgG positive median (IQR *)	Anti-HEV IgG negative median (IQR *)	p
HIV RNA viral load	2,030 (40 - 31,640)	17,900 (116 - 180,500)	0.132
ALT (normal < 50 IU/L)	25.0 (13.5 - 34.5)	22.0 (16.0 - 31.0)	0.788
AST (normal < 50 IU/L)	25.0 (18.0 - 28.5)	22.0 (18.5 - 28.5)	0.735
CD4 (cell/µL)	513 (379 - 651)	386 (237 - 604)	0.372
CD8 (cell/µL)	813 (735 - 1,890)	932 (719 - 1,240)	0.817
Lymphocytes(K/µL)	1,850 (1,500 - 3,100)	2,000 (1,520 - 2,495)	0.910

IQR - interquartile range.

Distribution of some biochemical values according to anti HEV IgG status is given in Table 3.

DISCUSSION

For many years, HEV infection was thought to be a self-limiting disease, especially in developing countries. Recently, it has been reported that chronic HEV infection with possible progression to cirrhosis has occurred among immunocompromised patients, including solid organ transplant recipients and patients with malignant hematological disorders in developed countries [8-10]. The aim of this study was to evaluate the sero-virologic prevalence of HEV infection in a group of HIV-infected patients. In our study, HEV seroprevalence was found to be 4% in HIV-positive patients. Studies have shown very different results regarding the incidence of HEV infection in HIV-positive patients. In these studies, anti-HEV IgG positivity was reported to be between 4.4% and 39.4% [11-15]. Several factors may play a role in the variability of HEV seroprevalence in HIV-positive individuals. Factors such as differences in socio-demographic structure of the countries, inadequate access to clean water, and immune status may have been affected. Further studies are needed to assess whether these differences in the results are related to the HEV genotype. We could not find a study evaluating the HEV seroprevalence in HIV-positive patients in our country. Studies have generally evaluated the incidence of HEV in the general population. In a recent systematic review, in which all these studies were evaluated, the studies conducted between 1980 and 2017 were evaluated and it was reported that the prevalence of HEV infection in healthy individuals in the general population and in some special groups was between 0% and 12.4% [7]. However, it is not possible to make a comparison with our results since their studies are not evaluated in terms of the frequency of HIV-positive individuals. Nevertheless, the prevalence of HEV reported in the general population is similar to the prevalence value of our study, so that the frequency of HEV infection in HIV-positive patients is not different from the prevalence of HEV infection in the general population. Nevertheless, the answer to the question of whether HIV infection causes HEV infection in humans remains unclear [16]. In studies conducted in non-endemic regions, it has been shown that HEV seroprevalence in HIV-infected individuals is not higher than in the control group [17]. Only in France, the United Kingdom, and the United States are there case reports of some acute and chronic HEV infections occurring in HIV-infected patients [18,19]. It has been reported that low CD4 count in HIV-positive patients is associated with higher anti-HEV IgG seropositivity [13]. In a study conducted in Nepal, a higher rate of HEV IgM positivity was observed in individuals with CD4 count > 200 [11]. There are also studies reporting the opposite of these studies. In a study conducted in Spain, HEV infection was investigated in HIV patients with advanced immunodeficiency (CD4 < 200 cells/mm^3) or in a group of selected patients with unexplained aminotransferase elevation [20]. In their studies, it was reported that only HEV-RNA patients were screened, no HEV-antibody examinations were performed, and no HEV viremia cases were detected from the screened patient group. According to this result, Madejon et al. concluded that the absence of HEV infection among HIV-positive patients with very low CD4 count did not increase the risk of chronic hepatitis E. In our study, no HEV positivity was detected in any patient with CD4 count below 200 supporting this result. The CD4 count of 5 patients with HEV positivity was found to be between 340 and 751. Nonetheless, factors such as methodological differences between studies and adequate sample size may have affected the inconsistency between the results.

CONCLUSION

In this study, anti-HEV IgG positivity was found to be 4% in HIV-positive individuals and was similar to the general population. No HCV and HBV co-infection was detected in any patient with HIV-HEV coexistence. HEV infections do not emerge as a priority among HIV-infected people, but HEV should also be investigated in

HIV-infected individuals' liver abnormalities of unknown etiology. Our study was one of the first cases to study HEV antibodies from the plasma of HIV-positive individuals by ELFA method. Comprehensive studies with more samples are needed for this new test method and for HEV studies in HIV cases.

Ethical Approval:
Obtained from the Ethics Committee of Sakarya University Faculty of Medicine with the number 71522473 /050.01.04/289 dated 12.12.2018.

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Declaration of Interest:
The authors declare that they have no conflict of interest.

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