Original Article

Seroprevalence of hepatitis E virus infection in acute leukemia patients with allogeneic transplantation

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Abstract

Aim: Hepatitis E Virus (HEV) infection is an emerging threat in allogeneic Hematopoietic Stem Cell Transplant (allo-HSCT) recipients. The aim of this study was to determine the prevalence of HEV infection in patients with acute leukemia before and after the transplantation.

Material and Methods: The study was carried out with the serum samples taken before and after the transplantation from patients (n=42) with acute leukemia, who were 4 months to 64 years of age and who had allogenic transplant at Gazi University Hospital. All samples were tested in terms of HEV-specific anti-IgG and IgM positive value by using ELISA kit.

Results: While 1 (2.3%) of the samples that were included in the study was positive anti-HEV IgG before transplantation, it was found negative after the transplantation (58th month). Positive anti-HEV IgM was not found before and after the transplantation in the collected samples. The average age of the seronegative group was determined as 30.8 ± 33.1 for male and female, respectively. There was no statistically significant difference in terms of anti-HEV IgM positivity and anti-HEV IgG positivity compared with seronegative cases of seropositive cases.

Conclusions: This study was conducted to determine the prevalence of HEV infection in allogeneic stem cell patients. With this study, the incidence and importance of HEV can be determined in these patients before and after transplantation. This study can also create an important epidemiological data for our country.

Keywords: Acute Leukemia; allogeneic transplant; ELISA; Hepatitis E virus; seroprevalence

INTRODUCTION

Globally, viral hepatitis infections are crucial health problems; and therefore, several studies are still ongoing to prevent and treat them (1,2). It is transmitted by the fecal-oral route, causing infection in the form of sporadic outbreak. Phylogenetic studies have shown that there are four main genotypes of HEV from 1 to 4. Genotype 1: Asian-African people; Genotype 2: Mexican-African people; Genotype 3: America-Europe human and pig; and Genotype 4: It has been reported as Asian human and pig strains. Genotypes 1 and 2 of HEV infect only humans. These genotypes are responsible for most waterborne human infections (3,4).

Hepatitis E virus causes significant mortality by creating fulminant hepatitis in women during the last three months of pregnancy, and results in 17.3% mortality in pregnant women (5).

Two-thirds of the patients with suppressed immune system against HEV infection tend to be candidates for chronic hepatitis, and require antiviral therapy. In light of the literature survey and case studies in recent years, ribavirin has been applied successfully to treat against chronic HEV infections (6).

Allogeneic Stem Cell Transplant (allo-SCT) is an effective treatment method in many hematological diseases and cancers. In this treatment, the patient is primarily subjected to high-dose chemotherapy (7).

Afterward, the stem cells collected from a healthy donor (sibling, relative or non-relative) are transferred to the patient. These stem cells enable the production of healthy blood cells in patients' bone marrow. The important hallmark that maybe highlighted in allogeneic stem cell transplantation is the "Graft versus Host Disease" (GvHD), which reflects the tissue differences between the donor and the patient (8).

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GvHD is a non-ignorable disease with drastic damage to the recipient's organs. Therefore, the highest compatibility between the patient and the donor is vigorously desirable. In conclusion, the principal survival amount after allogeneic transplantation is attributed to donor-recipient pairing, graft-host response, and improvement of the leukemia (9). In another aspect, the repetition of underlying disease and infections is the more often side effect of Allo-SCT (10,11). As an infection faced, bacterial infections, in particular, are frequent ones, while viral or fungal infections possibly protrude, and gastrointestinal tract, lung, mouth and skin infections are also seen (12). The available data about HEV infections in patients undergoing allogeneic stem cell transplants are restricted (10). To this end, the aim of this study is to determine the prevalence and clinical significance of HEV infection in allogeneic stem cell patients.

MATERIAL and METHODS

In this study, the serum samples were collected from 42 immunocompromised patients (Table 4) before and after the transplantation at Gazi University Hospital were used. The patients'age interval was between 4 months and 64 years. The data on allogeneic transplant profiles were thoroughly analyzed. All serums were tested in terms of HEV-specific anti-IgG and IgM positivity by employing the ELISA (Dia-Pro Diagnostic Bioprobes, Italy) method in line with the manufacturer's recommendations. All serum samples were kept at -20°C until tested for anti-HEV IgM and IgG in parallel.

ELISA protocol

Serum samples taken from the patients were taken out of the freezer (-80°C) and kept at room temperature for thawing and reaching the appropriate temperature. ELISA was performed by coating 96-well polystyrene microtitre plates. An appropriate number of microplates was used, and the first well from each plate was left blank. Negative and Positive controls were added to each plate; 200 ml sample diluent was distributed to all wells; and then 10 ml sample was added. Then, 50 µL assay diluent was added to all wells, and after 45 minutes of incubation at 37°C, the plate was washed 5 times with distilled water. Afterwards, 100 µL of enzyme conjugate, substrate buffered urea peroxide solution, and substrate B (Tetramethylbenzidine) were added respectively to each well. Reactions were stopped with H2SO4 (100µL), and optical density (OD) was read at 450 nm and 620-630 nm (Spectrophotometer, BioTek, USA). The cutoff value of each assay was calculated according to the manufacturer's instructions. The OD value for each sample was divided by the cutoff value of the assay.

Ethical approval

The present study protocol was reviewed and approved by Gazi University, Faculty of Medicine (Approval No. 181, on 29.09.2010).

Statistical analysis

In this study, the collected and measured data were expressed as numbers, percentages, average values, standard deviation, median, and minimum-maximum values. The Chi-Square Test was also used in this stud.

RESULTS

In the study, 42 serum samples before and after transplantation were included. The mean age of the patients was 13 (31%) in females, and 29 (69%) in males (between 4 months to 64 years old). According to age groups, 5 (11.9%) of the patients were between 4 months-17 years of age, 23 (54.8%) of the patients were between 18-35 years of age, and 14 of the patients (33.3%) were between 36-64 years of age (Table 1).

Table 1. The number and percent distribution of patients with acute leukemia in the mentioned ages (n = 42)		
Age groups (year)	Number	%
4 (month) – 17	5	11.9
18 – 35	23	54.8
36 – 69	14	33.3
Total	42	100

The serum samples collected from 20 patients had gender distribution as 14 males and 6 females within 1-10 month. The sera of the patients consisted of 14 (9 males and 5 females) taken in the 12-25 month period. The sera of the remaining 8 patients (6 males and 2 females) were collected in a period of 31-62 months (Table 2).

Table 2. Period versus gender before and after the transplant in the collected samples			
Total number	Female (n)	Male (n)	The collected samples after transplant (month)
20	6	14	1 – 10
14	5	9	12 – 25
8	2	6	31 – 62

Table 3. ELISA results according to the gender of the patients (n=42)			
Gender	Anti-HEV IgM Positive n (%)	Anti-HEV IgG Positive n (%)	Total (%)
Female	0 (0)	0 (0)	13 (100)
Male	0 (0)	1 (3.4)	29 (100)
Total	0 (0)	1 (2.3)	42 (100)
*Chi-Square=0.46 and p= 0.4979			

Anti-HEV IgM was not found positive before and after transplantation in the samples included in the study. While anti-HEV IgG assay results before the transplant in leukemia patients affected by allogeneic transplant seropositivity were found to be 1 (2.3%). The seropositive profile value after transplantation was found negative by the termination of 58 months' period. The seropositive

patient was 45 years old. When the seropositive case was compared with the seronegative cases, no statistically significant (p>0.05) difference was found in terms of anti-HEV IgM and anti-HEV IgG positivity (Table 3).

Statistically, the difference between anti-HEV IgM and anti-HEV IgG is not significant. In the Acute Myeloid leukemia patterns, anti-HEV IgG seropositive amount clearly witnessed as 1 (2.3%) (Table 4).

Table 4. ELISA results by diagnosis of patients (n=42)		
Diagnosis	Anti-HEV IgM Positive n (%)	Anti-HEV IgG Positive n (%)
AML n=19	0 (0%)	1(2.3%)
ALL n=9	0 (0%)	0 (0%)
HL n=9	0 (0%)	0 (0%)
MDS n=3	0 (0%)	0 (0%)
KML n=2	0 (0%)	0 (0%)
AML, Acute Myeloid Leukemia; ALL, Acute Lymphocytic Leukemia; HL, Hodgkin Lymphoma; MDS, Myelodysplastic Syndrome; and CML, Chronic Myeloid Leukemia		

In our study, anti-HEV IgG of the case with Acute Myeloid Leukemia (AML) diagnosis in terms of seasonal distribution of our data was found positive in the autumn as represented in Table 5.

Table 5. Seasonal distribution of the ELISA results of patients after transplantation (n = 42)		
Season	Anti-HEV IgM Positive n (%)	Anti-HEV IgG Positive n (%)
Winter N17	0(0)	0 (0)
Spring N6	0 (0)	0 (0)
Summer N6	0(0)	0(0)
Autumn N13	0 (0)	1 (2.3)

DISCUSSION

Viral hepatitis caused by hepatitis E virus is common all over the world. The prevalence of the disease potently depends on the socioeconomic level and geographic regions. In developing countries, more than 50% of the acute viral hepatitis are caused by either A and or B viruses (13,14).

In countries with insufficient environmental sanitation, since these outbreaks are capable of causing epidemics in many parts of the world, a significant proportion consists of sporadic hepatitis E cases. In accordance with studies on anti-HEV positivity, the results indicate that the repetition of HEV infection is increasing in Turkey (15).

In adverse anti-HEV seroprevalence studies carried out, the values were reported as 7% (in normal population), 11.7% (in general healthcare personnel), and 3.7% (in healthcare personnel in Adana, Antalya and Izmir). In addition, Aydın et al. also provided anti-HEV seroprevalence analyses that resulted in approximately 29% and 3% in Diyarbakir and

Trabzon (16). Taking a glance at other parts of the world, for instance, 10% (103/998) of the total 998 patients were influenced by anti-HEV positivity in Bangladesh (17). Again, in another study performed in Hanover (located in the north of Germany), the sequence of acute and chronic hepatitis E was exerted upon total 226 patients who underwent liver transplantation (18).

Recently, in another study conducted in Iran, the positive value of the anti-HEV IgG assays were obtained as 48 (9.7%), since the participants' (number is equal to 493) ages were altered as 40.98 ± 17.10 in 2019 (19).

In our study, anti-HEV IgG positivity (2.3%) was predominantly over the age of 40 years. When the results were compared, the occurrence of both Acute Lymphocytic and AML was nearly identical.

Regarding the outcomes, we found anti-HEV IgG seropositivity evidenced by the AML results. By taking diagnosis patterns into consideration, such statistical alteration in the reported positive values of anti-HEV IgM as well as anti - HEV IgG assays were not drastic.

Ross et al. considered the data obtained by the Child Cancer and Pediatric Oncology groups as a seasonal distribution standpoint, which disclosed prominent discrepancy between seasonal distribution versus ALL outcomes (ALL incidence reached peak point in summer months) (20).

In spite of the above mentioned results, positive values in anti-HEV IgG assays by the AML diagnostics were achieved in autumn in this investigation. As a result of this investigation, in the same manner globally, HEV infections were emphasized as a privileged factor in patients affected by acute leukemia aged between 4 months - 64years. Significantly, being young adults, living in crowded environments, using tap water for the purpose of drinking, as well as accommodating in areas with low socioeconomic levels progressively enhanced risk of HEV infection.

CONCLUSION

In our study, as in the world, hepatitis E virus infections have been shown to be an important factor in patients with acute leukemia between the ages of 4 months and 64 years.

Anti-HEV IgG was positive in 1 (2.3%) of the samples before the transplantation, the same patient was found negative after the transplantation (58th months later). Since the socioeconomic level of Ankara is high, no statistically significant differences were detected in terms of anti-HEV IgM positivity and anti-HEV IgG positivity by the gender of the subjects.

To date, very few studies have been conducted on the frequency of HEV in a similar patient group in the world and in our country; therefore, there is no detailed information about the frequency of HEV before and after transplantation in patients with leukemia. With this study,

the incidence and importance of HEV can be determined in these patients before and after transplantation. In addition, our study will create an important epidemiological data for our country.

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