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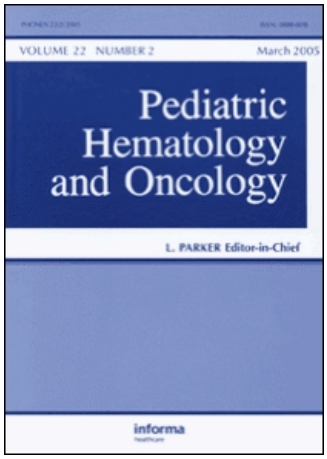

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TRANSFUSION-TRANSMITTED VIRUS PREVALANCE IN TURKISH PATIENTS WITH THALASSEMIA

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TRANSFUSION-TRANSMITTED VIRUS PREVALANCE IN TURKISH PATIENTS WITH THALASSEMIA

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□ *In hematology patients on chronic transfusion regimes, liver diseases are frequent, and mostly related to the agents transmitted by blood products and concominant iron deposition in liver. Besides hepatitis B (HBV) and C (HCV) viruses, new viral agents like hepatitis G virus (HGV) and TorqueTeno virus (TTV) are identified in these patients, although their association with any pathology or disease is not yet proved. In the present work, the authors studied the clinical importance of TTV in Turkish multitransfused patients with thalassemia. Forty-six healthy and 57 thalassemic patients were enrolled in the study. TTV was detected in serum samples by 3′-UTR nested PCR. Transaminase and ferritin levels, hepatitis B and C virus markers and number of transfusions were interpreted for possible association with TTV infection. As a result, TTV was detected in 63% of the thalassemia and 54% of the control patients. Prevalence of TTV infection, clinical features, laboratory data, and annual transfusion numbers of TTV-positive and -negative patients were not observed to be statistically significant. In conclusion, in Turkish patients with thalassemia, TTV infection cannot be considered as a risk factor for liver disease.*

Keywords healthy, HCV, thalassemia, TTV, Turkish children

Although routine screening of blood products for microbial agents transmitted by parenteral route markedly decreases the incidence of liver diseases

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related to those agents in patients on chronic transfusion regimes, hepatotrophic viruses like hepatitis B virus (HBV), hepatitis C virus (HCV), and non-A–G hepatitis viruses are still among major clinical problems of patients with hematological diseases. TorqueTeno virus (formerly known as transfusion transmitted virus, TTV) has been defined as a non-A–G hepatitis virus and was initially thought to be transmitted only by blood and blood products due to the fact that the index case was diagnosed as idiopathic post-transfusion hepatitis [1–3]. However, recent studies not only demonstrated the evidence of possible fecal–oral transmission, but also suspiciously high prevalence rates among healthy individuals [4–6]. After the discovery of the vast genetic diversity of the virus and variable sensitivities of detection methods used, the pathogenic role of TTV in hepatitis of known origin was questioned [7]. Currently, certain genotypes of TTV were thought to be more pathogenic and have the potential to cause liver injury, although convincing data about this concept is lacking [8, 9].

Studies focusing on TTV infection in Turkey is limited. Two particular reports about TTV infection in Turkish children with cryptogenic liver diseases and in Turkish adult thalassemia patients and healthy blood donors are of concern, where TTV was not found to be a risk factor in childhood cryptogenic liver diseases and TTV prevalence was high in thalassemic patients and healthy blood donors [10, 11].

This study was designed to reveal the prevalence of TTV in healthy children and thalassemic Turkish patients whose majority being children, to observe clinical and laboratory features of TTV infection, and to detect any significant feature in coinfection of TTV with HBV and HCV.

PATIENTS AND METHODS

A total of 57 patients with β-thalassemia major on chronic transfusion program were included in the present study. Controls were formed from a randomly selected group of 46 healthy individuals. The healthy individuals that comprised the control group of this study were either children brought to the outpatient pediatric clinic for mild illnesses like common cold or volunteer young adults. All individuals included in the control group were questioned for symptoms and signs of hepatitis and any prior transfusion history, surgery, and parenteral treatment and systematically examined by a pediatrician in the outpatient clinic. To be included in the study, informed consent was obtained from parents and/or patients.

For all thalassemic patients in the study, transaminase and ferritin levels, hepatitis B surface antigen (HBs), anti-HBs antibody, and anti-HCV antibody tests that were routinely done during last control visit and the number of transfusions along with demographic information were noted for statistical analyses.

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Detection of TTV DNA

Five milliliters of blood sample was collected in sterile EDTA tubes from thalassemia patients and healthy controls. DNA was extracted from 100 μL of each sample by using a Viral DNA Extraction Kit (Metis Biotechnology, Turkey) according to manufacturer's instructions. All samples were kept at −80°C until studied.

For the detection TTV DNA, a sensitive nested PCR protocol targeting 3′-UTR of the viral genome developed by Leary et al. was employed [5]. Primers B1 (sense, nt:3087–3110; 5′-GTGGGACTTTCACTTGTCGGTGTC–3′) and B2 (antisense, nt: 3392–3368; 5′-GACAAATGGCAAGAAGATAAAGGCC–3′) were used for a 50-μL reaction mix containing 10 μL of template, 2 mM $MgCl_2$, dNTPs, and Taq polymerase. For the second PCR, primers B3 (sense, nt: 3120–3141; 5′-AGGTCACTAAGCACTCCGAGCG-3′) and B4 (antisense, nt: 3362–3342; 5′-GAAGTCTGGCCCCACTCAC-3′) were added and $MgCl_2$ concentration was decreased to 1.75 mM (positions of the primers refer to TTV isolate TWH; GenBank Accession No: AB008394). Thermocycling consisted of 35 cycles of 30 s at 94°C, 45 s at 55°C, 45 s at 72°C after a denaturation step of 2 min at 94°C. A last polymerization step of 10 min at 72°C was also performed. The expected amplicons of 243 basepairs were seperated by electrophoresis on 2% agarose gel, and visualized under ultraviolet light after staining with ethidium bromide. Positive and negative controls were used for each reaction. Nucleic acid extraction, PCR, and electrophoresis were performed in different laboratories to prevent contamination.

Statistics

Chi-square test was used to analyze the difference of TTV prevalence between the groups. To test the difference of some clinical parameters between TTV-positive and TTV-negative groups, the student t test for parametric parameters and Mann-Whitney U test for nonparametric parameters were employed. We analyzed the difference between TTV- and HCV-positive, TTV- or HCV-positive, and TTV- and HCV-negative groups by a nonparametric Kruskal-Wallis test. Correlation analysis of TTV-HCV positivity and age was performed by Spearman's correlation tests. p values $<.05$ were considered as significant. The Statistical Package for Social Sciences (SPSS version 11.0) was used for statistical analysis.

RESULTS

Some features of thalassemia patients and controls are summarized in Table 1. Mean age of the thalassemia group (16 ± 6 years) was higher than that of controls (11 ± 5 years) ($p < .01$). There was no statistically significant

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TABLE 1 Some of the Findings of Thalassemic Patients and Controls

Features	Patients $n = 57$	Controls $n = 46$
Age (year)	16 ± 6 (3–30)	11 ± 5 (4–29)
Gender[a]		
Female	28 (49%)	22 (47.8%)
Male	29 (51%)	24 (52.2%)
ALT (IU L^{-1})	51 ± 48 (8–311)	NA
AST (IU L^{-1})	41 ± 28 (13–181)	NA
Number of transfusions/year	13 ± 3 (5–18)	0
Ferritin (ng/mL)	3045 ± 1990 (941–8500)	NA

Note. Data are given as means ± standard deviations (minimum–maximum). ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not available.
[a]This parameter is given as number (%).

difference in gender between groups ($p = .896$). TTV prevalence was 63% in the patient group and 54% in the control group, which was not statistically different ($p = .366$). All of the thalassemia patients were seropositive for anti-HBs antibody and were negative for HBs antigen. Some characteristics of TTV-positive and -negative thalassemia patients are given in Table 2. No statistically significant difference was observed between TTV-positive and -negative cases except that TTV-positive group was older ($p < .001$). Co-infection of TTV and HCV was detected in 8 of the patients. Some clinical features of these patients are listed in Table 3. When clinical and laboratory parameters of 8 TTV and HCV co-infected patients (14%), 30 patients positive for one of the viral agent (28 TTV and 2 HCV, 53%), and 19 patients negative for both of them (33%) were analyzed, no difference in alanine aminotransferase, aspartate aminotransferase, and ferritin levels ($p = .170$, .107, and .348, respectively), annual transfusion number ($p = .163$) was detected. Patients negative for both TTV and HCV (12 ± 4 year) were found to be younger than other 2 groups (17 ± 6 and 20 ± 3 years for patients positive either for TTV or HCV and patients positive for both) ($p = .001$). Co-infection of TTV and HCV was positively correlated with the age of the

TABLE 2 Some of the Findings in TTV-Positive and -Negative Thalassemic Patients

	TTV positive $n = 36$	TTV negative $n = 21$	p
Age (year)	16 ± 6	10 ± 5	<.001
ALT (IU L^{-1})	45 ± 30	61 ± 68	.722
AST (IU L^{-1})	39 ± 24	45 ± 35	.289
Ferritin (ng mL^{-1})	2770 ± 1862	3517 ± 2156	.155
Number of transfusions/year	13 ± 2	14 ± 3	.252
HCV			
Positive	8 (22%)	2 (10%)	.295
Negative	28 (78%)	19 (90%)	

Note. Data are given as means ± standard deviations (minimum–maximum). TTV, TorqueTeno virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

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TABLE 3 Summary of Thalassemic Patients with Co-infection of TTV and HCV

Case no.	Age (years)	Gender	ALT (IU L^{-1})	AST (IU L^{-1})	Ferritin (ng mL^{-1})	Number of transfusions/year
1	15	M	18	18	1234	12
2	23	M	62	37	5194	12
3	16	M	24	25	3379	13
4	23	F	45	37	941	17
5	18	M	82	64	983	18
6	20	F	88	75	1985	10
7	24	M	114	106	5722	12
8	18	F	105	84	1612	14

Note. TTV, TorqueTeno virus; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; F, female; M, male.

patient ($r = .352$, $p = .007$). HCV was present in 33% of the patients older than 18 years.

DISCUSSION

One of the major complications of blood transfusion is transmission of viral infections. Although recent developments in screening of HCV and HBV in blood products decreased the prevalance/incidence of hepatitis in patients on chronic transfusion occurring due to HCV and HBV, liver diseases related to secondary hemosiderosis and non-A–E viral agents occur commonly in thalassemic patients on hypertransfusion regimes. In 1997 TTV was defined as a virus causing hepatitis related to transfusion and was found to be responsible for 25–45% of the fulminant or chronic hepatitis of unknown origin [1–3, 8, 12]. The prevalence of TTV was also reported to be 69–94% in thalassemic patients on chronic transfusion regimes, 24–78% in hemophilic patients, and 2–60% in blood donors [1, 13–17]. TTV DNA was previously detected in Turkey in 14% of children with chronic liver diseases, 61% of adult thalassemia patients, and 52% of blood donors [10, 11]. In the present study we demonstrated that TTV was present in 63% of Turkish children with thalassemia and 54% of healthy individuals, confirming the findings of previous studies that TTV was not a risk factor for hepatitis and liver diseases of unknown origin in thalassemia patients. In the present study, the detection rate of TTV in thalassemic patients was as high as that of healthy controls, which also supports the view that high prevalence of TTV in thalassemia patients was related to and a reflection of high prevalence of TTV in healthy populations such as blood donors [11]. Thus, high prevalence of TTV in both healthy children and healthy blood donors supported the existence of another transmisson route of the virus other than the parenteral route [18]. A recent study demonstrated that TTV can be detected in samples like feces, urine, saliva, and breast milk, with a high number of copies in hepatocytes

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and mononuclear cells of the peripheral blood [4]. These results suggest the presence of possible fecal–oral, and/or vertical transmission of TTV [18].

Although the natural course of TTV is not fully understood due to lack of long-term follow-up of TTV-positive patients, it was shown that 86% of TTV-positive patients had chronic infection and TTV positivity was characterized by a mild and transient increase in alanine aminotransferase levels [19]. TTV was also reported to cause a subclinical liver disease, usually without markedly raised liver enzymes [1, 18]. In our study, alanine aminotransferase levels were 45 ± 30 IU L^{-1} and aspartate aminotransferase levels were 39 ± 24 IU L^{-1} in thalassemic patients infected with TTV and not significantly higher than TTV-negative individuals. The finding that TTV-positive and -negative groups also had similar clinical and laboratory features suggested that presence of TTV infection did not affect the severity of liver disease.

In a study of 68 adult thalassemic patients, co-infection of HCV and TTV was related to higher necroinflamatory activity in liver histologic preparations than those in isolated HCV infection [13]. These findings were not confirmed in our study where no difference in clinical and laboratory data of HCV/TTV co-infected persons could be demonstrated. However, co-infection seemed to occur in the older age group which might again reflect an increased number of total transfusions or exposure to the virus as a result of some undefined environmental factors.

The nested PCR protocol used in our study for TTV DNA detection was developed by Leary et al. and targets 3′-UTR (untranslated) segment of the viral genome [5]. Since it was reported to be a very sensitive assay that detects TTV strains not only from most of the human sera used in the study, but also from nonhuman sources, this set was chosen to be used for our study that required increased sensitivity for all genotypes/subtypes of the virus. Other approaches for TTV detection are targeting the 5′-UTR by single round, nested, which present close/comparable results [7, 20]. Although degenerated primers that target ORF1 were also reported to achieve good sensitivity results, PCR protocols for this region is usually accepted to be specific for certain genotypes of TTV [20, 21]. Since viral DNA amplification by PCR is the most frequent detection method for TTV, sensitivities of different primers used for PCR must be taken into consideration when comparing results from different studies. It is surprising to note that even by using ORF1 PCR, no difference in detection rates of TTV was demonstrated in other studies from Turkey for thalassemic or cryptogenic hepatitis cases and controls [10, 11]. This implies a similar genotypic distribution of TTV in patients and controls not only in our study but also in reports mentioned above, which, again, suggests a high prevalence and frequent transmission in the Turkish population.

In conclusion, TTV is not found to be a risk factor for liver diseases in Turkish multitransfused thalassemia children. High TTV prevalence is possibly a reflection of high prevalence in healthy Turkish children. New, larger, and long-term follow-up studies will demonstrate the role and course

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of TTV in thalassemic children and other multitransfused patients. For a better understanding of possible pathogenic role of TTV in liver diseases, genotypes of TTV should also be studied.

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