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Original Article

Transfusion-transmitted virus DNA in serum, tear and aqueous humour of patients undergoing cataract operation

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ABSTRACT

Purpose: Transfusion-transmitted virus (TTV) is a novel non-enveloped, single-stranded DNA virus with unclear pathogenesis throughout the world. Many studies were conducted to determine this virus in various body fluids and different primer sets have been tested for accurate diagnosis. This study aimed to collect data on the prevalence of TTV in serum, tear and aqueous humour of patients undergoing planned cataract surgery and to determine efficacy of three different polymerase chain reaction (PCR) techniques.

Methods: A total of 72 specimens (24 each of serum, tear and aqueous humour specimens) were collected from 24 patients (11 male and 13 female) having age-related cataract. The patients did not have any other ocular pathology. TTV DNA was investigated by three different PCR methods: a seminested PCR performed with Okamato's primers, a one-step PCR performed with degenerative Takashi's primers and a commercial real-time PCR system.

Results: TTV DNA was detected in 20 (83.3%) of the 24 serum specimens by the one-step PCR and real-time PCR system. However, seminested PCR yielded a positivity rate of 25%. TTV DNA positivities of the one-step PCR and the real-time PCR system were 33.3% and 66.6% of the 24 tear specimens, respectively. Seminested PCR did not yield positive result in these specimens. From aqueous humour specimens, TTV DNA was detected in 3 (12.5%) of the 24 specimens only by the real-time PCR. TTV DNA positivity of seminested PCR was significantly low in all specimens.

Conclusions: TTV DNA was detected in serum, tear and aqueous humour of patients undergoing cataract surgery, supporting the idea that this virus can be detected almost all of the body fluids but at different rates under various PCR conditions and primer sets. Using commercial real-time PCR significantly increased the TTV DNA positivity.

Key words: aqueous humour, PCR, serum, tear, TTV.

INTRODUCTION

In 1997, a novel DNA virus, transfusion-transmitted virus (TTV), was first isolated in a Japanese patient with post-transfusion hepatitis of unknown aetiology.[1] It was tentatively designated in Anellovirus. TTV is a non-enveloped, single-stranded DNA virus measuring 30–50 nm.[2] TTV genome sequence is heterogeneous and reveals the existence of six different genotypes and several subtypes.[3]

Despite the ubiquitous nature of the virus, there have been numerous studies attempting to identify a pathogenetic role for TTV.[4,5] At first, TTV was suspected to cause of non-A-G hepatitis, but following studies indicate that it does not have an aetiological role in acute hepatitis. Up to now, TTV could not be blamed in any specific human pathology. It appears that viraemia is a frequent occurrence in apparently healthy individuals worldwide. Longitudinal studies showed that TTV frequently persists in the plasma of infected subjects for years.[6,7]

Even though initially it was thought that this virus was transmitted by only blood and blood products, recent studies documented the alternate transmission routes such as faecal-oral, transplacental and sexual route. TTV was detected in serum, semen, saliva, stool, liver and cerebrospinal fluid of different patient groups.[8–11] To our knowledge, there are no data about its presence in aqueous humour specimens. The present study was aimed to obtain data on the prevalence of TTV in serum, tear and aqueous humour of patients undergoing planned cataract surgery and to compare efficacy of three different polymerase chain reaction (PCR) methods on TTV DNA positivity.

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METHODS

Patients

A total of 24 (11 male and 13 female) patients with age-related cataract were included in the present study. These patients did not have any other ocular pathology. None of the patients had any immunosuppressive condition, or opportunistic infection. To be included in the study, informed consent was obtained from all participants. The study was approved by the ethical committee of Inonu University School of Medicine and carried out in accordance with the Declaration of Helsinki.

Sample collection and preparation

Serum and tear samples of patients were collected preoperatively by ophthalmologist (SE and SD). Tear samples were obtained from conjunctiva sac with a haematocrit tube after conjunctival stimulation with a cotton tip. Blood samples were collected in vacuum tubes. All surgeries were performed by two surgeons (SE and SD). The surgery was performed under topical anaesthesia. After periorbital skin cleaning with povidone-iodine (10%), the skin and eye lashes were draped. After that, povidone-iodine (5%) was applied to conjunctiva, and it was washed away with fortified balanced salt solution (BSS Plus). At surgery, first two side-ports were prepared with 20-gauge stiletto and 0.1 mL of aqueous humour was aspirated with an anterior chamber cannula connected to insulin injector. After that, surgeries were performed according to surgeon's preference technique without any complication. A total of 72 specimens (24 serum, 24 tear and 24 aqueous humour specimens) of the 24 patients were stored at −80°C till DNA extraction.

Virus detections

DNA extraction

DNA was extracted from 200 μL of each specimen by using DNA extraction kit (Qiagen Ltd, Crawloy, UK). Purified DNA was dissolved in 50 μL of elution buffer.

Amplification of TTV DNA

Transfusion-transmitted virus DNA was investigated by using two in-house PCR systems and a commercial real-time PCR kit.

In-house PCR for TTV DNA detection. Two in-house PCR sets, seminested PCR and one-step PCR, were performed to investigate TTV DNA in a total of 72 specimens (24 serum, 24 tear and 24 aqueous humour specimens). Seminested PCR was performed with the Okamoto's primers generated from the N-22 regions of the open reading frame (ORF) 1 of the TTV genome groups 1 and 2.[3,12] The first round of the seminested PCR was carried out with 5 μL of the extracted DNA using 10 pmol primer NG059 (sense: 5′-ACA GAC AGA GGA GAA GGC AAC ATG-3′) and 10 pmol primer NG063 (antisense: 5′-CGT GCA TTT TAC CAT TTC CAA AGT T-3′) for 35 cycles at 94°C for 30 s, 58°C for 30 s, 72°C for 30 s with additional 7 min for a final extension step at 72°C. The second round of the seminested PCR was performed with 2 μL of the product of the first-round PCR using another sense primer NG061 (5′-GGC AAC ATG TTA TGG ATA GAC TGG-3′) and primer NG063 for 25 cycles with the same conditions. The one-step PCR was carried out with 5 μL of the extracted DNA using 30 pmol each of Takahashi's degenerative primers derived from 5′ non-coding region;[13] primer T801 (sense 5′-GCT ACG TCA CTA ACC ACG TG-3′) and T935 (antisense 5′-CTB CGG TGT GTA AACT CAC C-3′, B: mixture of G, C and T). Amplification conditions were as follows: initial denaturation at 95°C for 9 min and 55 cycles at 95°C for 20 s, 60°C for 20 s, 72°C for 30 s with an additional extension step at 72°C for 5 min. Amplification product of the Okamoto's (272 bp) and Takahashi's (204–185 bp) primers were electrophoresed in 2% agarose gel, stained with ethidium bromide and visualized under UV light. Distilled water was used as a negative control for in-house PCR. All standard precautions were taken to avoid sample to sample contaminations and PCR product carryover.

Real-time PCR for TTV DNA detection. A commercial real-time PCR system was performed using the Fluorion TTV-QLS 1.0 (Iontek, Istanbul, Turkey) and the IQ 5 sequence detector system (Biorad, CA, USA). PCR mixture contained QuantiTect SYBR PCR mix with HotStarTaq DNA polymerase and detection reagents. The assay is targeted to amplify a highly conserved segment of the 5′ untranslated region of the TTV genome and it is able to detect all of the known TTV genotype. The specificity of PCR was demonstrated by melting curve analysis (88 ± 2°C). Real-time PCR system has its positive and negative control in the kit's box.

RESULTS

The mean age of patients was 64.3 varying between 45 and 85 years. Eighteen patients (75%) had associated systemic problems. The most common systemic illness was hypertension (50%) followed by diabetes mellitus (33%). Two patients had hepatitis B virus (HBV) infection; one of them had related chronic hepatic insufficiency. Also four patients had positive serological marker for HBV vaccination (Table 1).

Seminested PCR performed with the Okamoto's primer detected TTV DNA in 6 (25%) serum specimens of the 24 patients. Whereas when one-step PCR with Takahashi's primers and real-time PCR system were used, TTV DNA was detected in 20 (83.3%) serum specimens. TTV DNA was found in 8 (33.3%) and 16 (66.6%) of 24 tear specimens by the one-step PCR and real-time PCR, respectively. The positivity rate of the seminested PCR was significantly lower than those of the real-time PCR and one-step PCR. Seminested PCR did not yield positive result in tear specimens. TTV DNA was detected in 3 (12.5%) of the 24 aqueous humour

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Table 1. Demographic data, associated systemic disease profile and TTV results of patients undergoing cataract operation

No.	Age	Gender	Associated systemic disease	Cataract type	Serum TTV DNA	Tear TTV DNA	AH TTV DNA
1	60	M		N	–	–	–
2	71	F	Breast carcinoma	M	+	–	–
3	67	F		N	+	–	–
4	76	F	DM, HT, CHD	N	+	–	–
5	60	M	DM, CHD	N	+	+	–
6	70	F	HT	N	+	+	–
7	66	M	DM, HT	N & P	+	–	–
8	58	M	DM, HT	N	+	+	–
9	80	F	HT, CHD	N & P	+	+	–
10	45	M	DM, CRF	N	+	+	–
11	47	M		N & P	+	+	–
12	46	F	HT	P	+	+	–
13	47	F	CHD	N	+	+	–
14	74	M		N & C	+	+	–
15	55	M		M	+	+	–
16	80	F	HT, CHF	M	+	+	+
17	64	M	DM, HBV (+), hepatic insufficiency	P	+	+	–
18	53	F	HT	N & P	+	+	+
19	57	M	DM, HT, HBV (+)	N & P	+	+	+
20	76	F	HT	N & P	–	–	–
21	69	M	DM, HT, CHD	N	–	–	–
22	68	F	HT	N	–	–	–
23	70	F		N	+	+	–
24	85	F	DM	N, C, P	+	+	–

AH, aqueous humour; C, cortical; CHD, chronic hepatic disease; CHF, congestive heart failure; CRF, chronic renal failure; DM, diabetes mellitus; F, female; HBV, hepatitis B virus; HT, hypertension; M (gender), male; M (cataract type), mature; N, nuclear; P, posterior subcapsular; TTV, transfusion-transmitted virus.

specimens only by real-time PCR system. All the patients having TTV DNA in their tear and aqueous humour specimens were also positive for TTV DNA in their sera. Moreover, all patients having TTV DNA in aqueous humour were also positive in their tear specimens. Comparison of the TTV DNA results with some demographic and clinical findings of the patients was shown in Table 1.

DISCUSSION

Since the discovery of TTV, many investigations have been carried out to highlight its epidemiological, clinic and pathogenic properties. Possible relation of the virus with different clinical conditions may help to understand its pathogenesis. The current study showed the presence of TTV DNA in different body fluids of the patients undergoing cataract operation. We found that 83.3% of the patients undergoing cataract operation had TTV DNA in their sera, and this positivity was significantly higher than those obtained from tear (66.6%) and aqueous humour (12.5%) specimens of the same patients. TTV DNA was only detected in tear and aqueous humour of the cases having TTV DNA in their serum. In agreement with our results, previous studies have also observed the highest TTV DNA positivity in sera and discrepancy in the prevalence of TTV between tissue and serum specimens.[8,14,15]

Transfusion-transmitted virus DNA positivity in serum of healthy subjects varied from 2% to 98% in both developed and underdeveloped countries.[8–11,16–18] High genomic variability of the TTV virus may be the leading cause of such a big variation in the prevalence rates reported from different studies. Takahashi *et al.*,[13] Ozyurek *et al.*[17] and Desai *et al.*[19] documented variable results from the same patients with different primers. In order to get maximum positive results, the use of more than one primer pairs is recommended.[19] The low sensitivity of Okamoto's primers had been eliminated by using the degenerative Takahashi's primers.[13] Okamoto's primer can only detect restricted number of genotypes, but Takahashi's primers are able to detect all known genotypes of TTV[20] and the sensitivity of Okamoto's primer derived from ORF1 regions was 10–100 times lower than that of the primers derived from 5′ non-coding region.[13] In parallel to these data, we obtained considerable high positive results by the one-step PCR with Takahashi's primers and these PCR results were in more agreement with real-time PCR system than those of the seminested PCR performed with Okamoto's primers. Similar to our results, TTV DNA had been detected in 92% of the healthy persons with the Takahashi's primers, but it was positive just in 23% of the subject by the PCR with Okamoto's primers.[13] As it was expected, we found that the sensitivity of the real-time PCR was significantly higher than those of one-step PCR for all tested specimens

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and seminested PCR for tear and aqueous specimens. This situation can be resulted from two reasons. First, the level of TTV DNA in the specimens that yielded positive results by real-time PCR but negative results by one-step PCR and seminested PCR might be lower than those of the detection limit of the last two PCR protocols. Another speculation for the low sensitivity of seminested PCR may be because of the variety of genotypes. From the kit instruction we can understand that the real-time PCR is organized to detect all genotypes and, as it was indicated earlier, Takahashi's primers used in one-step PCR are able to detect all TTV genotypes. However, Okamato's primers generated from ORF1 region cannot detect all genotypes in group 2 which is one of the most commonly detected TTV genotypes in Turkey[21,22] and many other genotypes in groups 3 and 4 which were less frequently found in our neighbour city population.[22] It is more probably that the specimens showing TTV DNA positivity by real-time and one-step PCR, and negativity by seminested PCR have TTV genotypes that cannot be detected by Okamato's primers.

As mentioned previously, TTV infection was first reported in a hepatitis patient with an unknown aetiology and following publications have been documented about its high frequency in hepatitis patients.[9,23] In agreement with previous data, there were two patients with HBV infection in our study group and both of these also had TTV DNA positivity.

In conclusion, although the frequency of TTV in different body fluids and its transmission via blood and blood products have been well documented, this is the first study to report the intraocular presence of this virus. Although aqueous humour is a unique body fluid, tear can be a transmission route. Moreover, presence of TTV in aqueous humour may be one of the reasons of intraocular inflammation with unknown aetiology. Our future study will be conducted to explore this theory.

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