

Original Article

The presence of Torque teno virus in chronic obstructive pulmonary disease

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Abstract: Torque Teno Virus (TTV) has been identified as transfusion-transmitted virus in humans, initially. Although TTV viremia is extremely common in the general population worldwide, there is no direct causal evidence linking TTV infection to specific clinical manifestations. Our hypothesis was that TTV might play a role in Chronic obstructive pulmonary disease (COPD) by inducing inflammatory mechanisms previously identified. The study was conducted on 57 COPD patients and 39 healthy control groups. COPD patient groups included: the patients (n:20) with exacerbation needed noninvasive ventilation, the patients (n:19) who received only medical treatment, and the invited patients (n:18) for outpatient control. Serum samples were collected from patients and voluntary blood donors. TTV DNA quantification was carried out with a real time PCR by the hybridization probe system and viral load was interpreted through the crossing point value. TTV DNA was detected in the majority of both patients and healthy control groups. The prevalence was 94.4% (17/18) in patients for outpatient control, 94.7% (18/19) in patients who received only medical treatment, 100% (20/20) in patients with exacerbation needed noninvasive ventilation and 84.6% (33/39) in healthy controls. This difference was not statistically significant. However, CP values was statistically different in all the patient groups from the control group. TTV DNA prevalence was higher in patients than healthy individuals. More interesting thing, viral load was highest in the patients with exacerbation needed noninvasive ventilation. As a result, TTV may be associated with COPD and the severity of it.

Keywords: Torque teno virus, chronic obstructive pulmonary disease

Introduction

Torque teno virus (TTV) is a small nonenveloped virus. Its genome is a circular single-stranded piece of DNA of a negative polarity. TTV has been identified as transfusion-transmitted virus in humans the first description of TTV in 1997 by Nishizawa et al. [1]. TTV lacks sequence homology with any known viruses. It was classified under the family Anelloviridae and renamed *Torque teno virus* in 2009 [2]. Although TTV viremia is extremely common in the general population worldwide, there is no direct causal evidence linking TTV infection to specific clinical manifestations. On the other hand, the high prevalence of TTV infection has been found in some clinical manifestations such as liver diseases, acute respiratory diseases, renal diseases, AIDS and drug users in

recently studies [3-7]. However, there are also reports indicating the high prevalence in healthy individuals [8]. TTV may persist and cause chronic viremia [9]. The viral genome has been demonstrated from many tissues and secretions. The airways might be the primary route of transmission [10]. However, a causal relationship to clinical symptoms has not been demonstrated clearly.

Chronic obstructive pulmonary disease (COPD) are very common inflammatory diseases of the airways. Currently 64 million people have COPD and 3 million people died because of COPD. According to WHO estimates, it will be ranked the third cause of mortality in the world by 2030 [11]. COPD are characterized by chronic airway inflammation and airflow limitation. The inflammation develop, generally by inhalation of harm-

ful substances, such as smoking. There are accumulation of neutrophils, CD8-positive cytotoxic cells, and activated macrophages, in the process of inflammation. Acute worsening of symptoms and lung function, which often results in respiratory failure is a so-called "exacerbation, and respiratory viral infections are a major cause of exacerbations in patients with COPD. So that, virus infections have been implicated for about half of exacerbations of COPD in recent studies [12]. It is well known that the cigarette smoke activates innate immune cells by Toll-like receptors (TLRs), triggering the production of cytokines. Recently, Toll-like receptor 3 (TLR3) has been demonstrated to react to the viruses. The researchers suggested that this pathway may be a therapeutic target for viral-induced exacerbations of chronic obstructive pulmonary disease [13]. It has been shown TTV has the potential to stimulate and co-stimulate inflammatory responses via toll-like receptor 9 (TLR9) [14]. Interestingly, the recently published report showed that, cigarette smoke increases TLR9 expression and induces cytokine production from CD8+ T cells in chronic obstructive pulmonary disease [15]. We thought a hypothesis inspired by all these informations: can the persistent TTV infection cause respiratory tract inflammation and COPD? Actually, a positive correlation has been shown between other respiratory diseases such as asthma, bronchiectasis and idiopathic pulmonary fibrosis with high TTV concentrations, in some studies [16]. However, our study is the first research concerning the relationship between COPD and TTV. What does the prevalence of TTV in COPD patients and healthy individuals? Is there a relationship between the presence or load of TTV and severity of the disease? It was investigated for answers to these questions in this study.

Material and methods

This study was performed at the Hospital of Department of Medical Microbiology, Meram Medicine of Faculty, Necmettin Erbakan University, Konya, Turkey in April 2013 and August 2013. The study was conducted on 57 COPD patients and 39 healthy controls. The patient group attended from the chest disease clinic and, the control group included voluntary blood donors from the blood center of our hospital.

This protocol has received ethics approval from the Necmettin Erbakan University Medical School Human Research Ethics Committee. Informed consent was obtained from all the individuals at the initial presentation. The individuals who made blood transfusion previously not included in the study. COPD patient groups included: the patients (n:20) with exacerbation needed noninvasive ventilation (COPD-1), the patients (n:19) who received only medical treatment (COPD-2), and the invited individuals (n:18) for outpatient control (COPD-3). The patients having both anti-hepatitis C virus and anti-HIV antibodies not included in the study. The voluntary blood donors were required to be in good health, defined as the absence of chronic diseases, HIV, hepatitis B and hepatitis C infections.

Serum samples were collected from patients and voluntary blood donors and evaluated in the department of Medical Microbiology. Samples were stored at -70°C, until use. Total DNA was purified from 200 µl of serum using the High Pure Viral Nucleic Acid Kit (Roche Diagnostic, Mannheim, Germany) and the elution volume was set to be a final 50 µl. TTV DNA quantification was carried out with a real time PCR by the hybridization probe system which consists of two fluorescently labeled oligonucleotides. To amplify most TTV strains, specific oligonucleotide primers were employed derived from the ORF2 as conserved region of TTV. Previously published sequence of TTV genome was utilized for deciding the sequence of oligonucleotides [17, 18]. The hybridization probes were labeled with LC (LightCycler) Red 640 at the 5' end (LC probe, acceptor probe) and with fluorescein at the 3' end (FL probe, donor probe), for detection of the target sequence (Lightcycler Faststart DNA Master Hybprobe, Roche Diagnostic, Mannheim, Germany). For detection of the internal control, hybridization probes were labeled with LC red 705 at the 5' end and with fluorescein at the 3' end. It was decided to forward primer (5'-CCGAATGGCTGAGTTTTCCA-3', position 103 to 122), reverse primer (5'-TTTTT-CAGAGCCTTGCCCATAG-3', position 259 to 238), FL probe (5'-CGAATTGCCCTTGACTTCG-GTGTG-3', position 219 to 195) and LC probe (5'-AACTCACCTTCGGCACCCGCCCTC-3', position 192 to 169) with reference to AB0008394 GenBank accession ID. On the other hand, V00618 gene locus of *E.coli* was used as

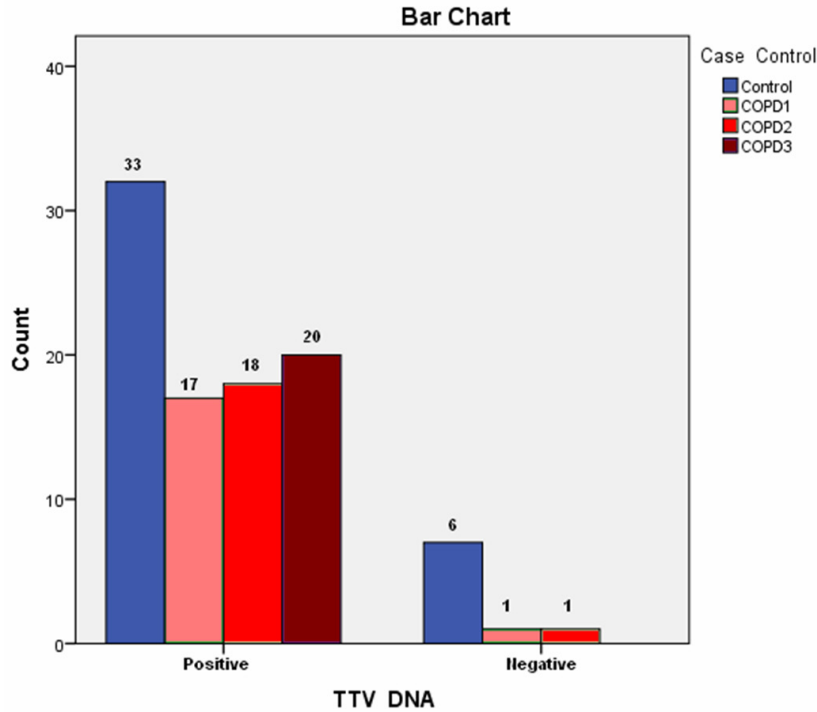


Figure 1. The presence of TTV DNA and clinical characteristics of the subjects.

Table 1. The CP values for PCR positive results in all groups

Groups	N	Mean (CP)	Standard deviation	Minimum	Maximum
Control	33	36.818	3.459	32.54	48.23
COPD-1	17	34.647	2.548	30.00	38.50
COPD-2	18	34.111	1.906	30.02	38.89
COPD-3	20	32.850	1.872	29.81	36.85

hybridization probe for detection of the internal control (Lightcycler Faststart DNA Master Hyb-probe, Roche Diagnostic, Mannheim, Germany) [19].

The PCR master mix was prepared to a final volume of 15 µl, and a 5 µl extracted sample was added to the master mix in LC glass capillary, as described by Koidl et al. [20]. Real time PCR was performed on the LightCycler instrument (Roche). The cycling protocol was run as follows: one cycle of 95°C for 7 min followed by 65 cycles consisting of denaturation for 1 s at 95°C, annealing for 10 s at 64°C, and elongation for 25 s at 72°C. After the final cycle, the melting curve was started at 50°C for 1 min and the thermal chamber temperature was slowly (0.2°C/s) raised to 85°C and the fluorescence was measured stepwise. The capillaries were then cooled for 2 s at 40°C. Fluorescence curves were analyzed with the LC software (ver-

sion 3.5.3), as described by Koidl et al.

Statistical analysis

Our data were analyzed using SPSS 16.0 statistical software. The descriptive statistics as the mean and standard deviation, and the categorical variables information as frequency were given. Chi-square analysis was used for the relationship between positive and negative categories in terms of TTV DNA and COPD. The distribution of variables was analyzed with the Shapiro-Wilk test and Kolmogorov-Smirnov. One-way ANOVA was used for group comparison. Dunnett’s C test method was preferred for pairwise comparisons with control group. Tamhane T2 were used as a post hoc tests for group differences in COPD groups.

Results

Mean age was 51.8 ± 8 years (36 males) in the patients group, and was 37.4 ± 5 years (36 males) in the control group. The presence of TTV DNA and clinical characteristics of the subjects are shown in **Figure 1**. TTV DNA was detected in the majority of both patients and healthy controls. The prevalence was 94.4% (17/18) in patients for outpatient control, 94.7% (18/19) in patients who received only medical treatment, 100% (20/0) in patients with exacerbation needed noninvasive ventilation and 84.6% (33/39) in healthy controls. This difference was not statistically significant, (p>0.001). The CP values for PCR positive results in all groups shown in **Table 1, Figure 2**. There was significant difference for CP value among the groups, (p<0.001). This difference was found between the control group with all three patient groups. Whereas there was no difference among the three COPD groups.

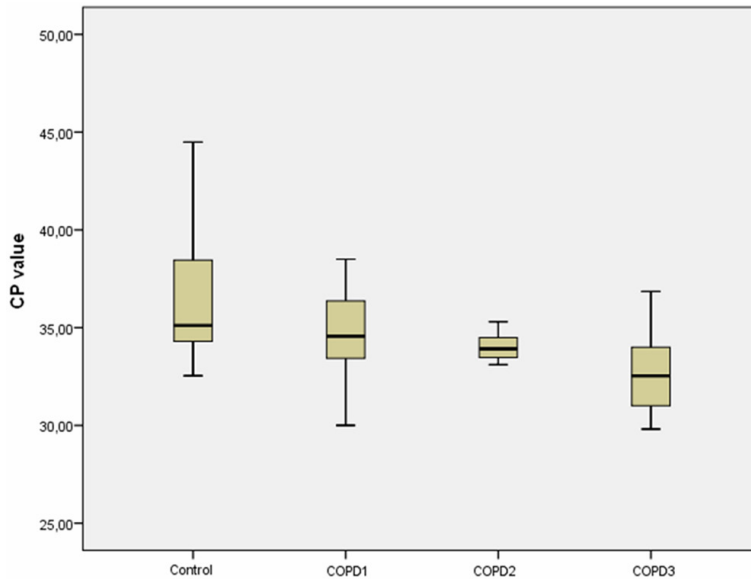


Figure 2. The CP values for PCR positive results in all groups.

Discussion

TTV may persist and cause chronic viremia [9]. It has been demonstrated from many tissues and secretions. Possible association with post-transfusion hepatitis, respiratory disease, and hematopoietic malignancies has been reported [1, 21, 22]. However, a causal relationship to clinical symptoms has not been demonstrated clearly. It is known that the airways might be the primary route of transmission, and TTV is possibly able to replicate in airway tissues [10]. On the other hand, some studies showed that TTV concentrations have a positive correlation with disease such as asthma, bronchiectasis, idiopathic pulmonary fibrosis, and some hypotheses such as a direct viral effect or be mediated by inflammatory processes that predispose to virus replication have been constructed to explain this situations [16]. Chronic airway inflammation is the major mechanism for of COPD and it is caused generally by inhalation of harmful substances, such as smoking. The key role of TLRs in the occurrence of inflammation has been shown repeatedly in many studies. "Cigarette smoke increases TLR9 expression and induces cytokine production from CD8+ T cells in chronic obstructive pulmonary disease" and "TTV has the potential to stimulate and co-stimulate inflammatory responses via TLR9" were specified in the recently. When planning this study, our hypothesis was that TTV might

play a role to COPD by inducing inflammatory mechanisms previously identified. TTV virus viremia and viral load could be an important indicator for us. On the other hand, viral load determination was very important, because the prevalence of the virus was high in healthy individuals in many studies. In our study, viral load was interpreted through the CP value. Our study is the first research concerning the relationship between COPD and TTV.

TTV DNA was detected more frequently among patients with COPD than the healthy group, in our study. The prevalence of TTV in healthy individuals, and variety risk groups

(such as haemodialysis patients, hepatitis patients) were reported in the range of 1-75% in variety studies [8, 23, 24]. The prevalence rate for healthy blood donors in a study conducted in our country has been found to be 16.8% by PCR targeted UTR and ORF1 regions of TTV25. Another study conducted in our country, TTV DNA has been detected via nested N22, nested 3'-UTR and 5'-UTR PCRs, and the prevalence was reported as 47.5% in healthy children26. TTV was investigated by 3'-UTR nested PCR in a study published in 2006, and it was detected in 63% of the thalassemia and 54% of the control groups [27]. In our study, higher prevalence of TTV in both healthy and patient groups is noteworthy. Although, the results of the above studies obtained by various PCR target region and in different age groups, higher prevalence in this study is an important finding. The seroprevalence of TTV were higher in patients than healthy individuals in our study, this difference was not statistically significant, Nonetheless, this higher prevalence in patients groups may be associated with the presence of COPD or disease clinic.

In order to better understand the causal relationship, we should know that "how is the viral load in patients and in healthy individuals?". So that, this claim could highlight more clearly. Thus, the viral load determined by CP value and exciting results emerged. So much so, the mean values of CP were lower in patients than

in healthy individuals, so viral loads were higher in patients. Moreover, there was a positive correlation between viral load with the severity of the disease. These differences were statistically significant and, all the patient groups were different from the control.

To date, we know those: viral infections are the most important cause of acute COPD exacerbations and, chronic infection of the airways amplify and perpetuate chronic inflammation in stable COPD. On the other hand, it is controversial these viruses play role of stable disease. The findings from previous studies demonstrate that at least one TTV isolate has the potential to stimulate and co-stimulate inflammatory responses.

Our findings demonstrate for the first time that TTV may be associated with COPD and/or the severity of it. We believe that the results of our study will inspire to next studies in which new evidence on this issue will be investigated and new study will support our conclusions.

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Disclosure of conflict of interest

None.

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