TTV and autoimmune hepatitis

**Transfusion transmitted virus and autoimmune hepatitis. Is there any association?**

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The pathogenesis of autoimmune hepatitis remains elusive; however, multiple agents have been implicated as triggers of this disorder. A novel deoxyribonucleic acid (DNA) virus, named transfusion-transmitted virus (TTV) was identified in 1997 in Japan, and has been implicated as a candidate virus of cases of unexplained hepatitis. We conducted a case-control study to evaluate whether TTV has a role in autoimmune hepatitis or not. Ninety patients with autoimmune hepatitis type-1 who had been registered in the Research Center for Gastroenterology and Liver Disease (RCGLD) between June and September 2003, were enrolled in this study. The diagnosis of autoimmune hepatitis was confirmed according to international diagnostic scoring system. Blood samples were taken before and during treatment in different individuals. The samples were centrifuged and the serum stored at 70°C within 4 hours of collection. As a control group, sera of 90 healthy staff that had been registered as mentioned above, and matched in sex and age were also included in this study. They had no evidence of hepatitis B or C virus infection. Also, serum transaminases were tested in all of them, and no one had elevation of liver enzymes.

Serum sample from all patients was stored at 70°C until assays. Deoxyribonucleic acid was extracted from 250ul serum with proteinase K and sodium dodecyl sulfate. Transfusion-transmitted virus DNA was amplified by super taq DNA polymerase (England) with semi nested primers derived from the sequence of ORF1. The specific primers used for polymerase chain reaction were as follows: NG059: 5-ACA GAC AGA GGA GAA GGC AAC ATG-3 [nucleotide position 1900-1923 in TA278 (accession number: AB 008394)]. NG061: 5-GGC AAC ATG YTR TGG ATA GAC TGG (Y=T or C R=A or G, nucleotide position: 1915-1938). NG063: 5-CTG GCA TTT TAC CAT TTC CAA AGT T-3 (nucleotide position: 2161-21805).

The first round of PCR was performed with the forward primer NG059 and the reverse primer NG063 for 15 minutes at 95°C followed by 35 cycles, consisting of denaturation for 30s at 94°C, annealing for 45s at 60°C extension for 45s at 72°C using a personal thermocycler (Eppendorf, Germany). The second round of PCR was performed with the sense primer NG061 and the antisense primer NG063 for 25 cycles, under the same condition. The amplified products were analyzed by electrophoresis on 1.5% agarose gels, stained with ethidium bromide and observed under ultraviolet light.

There were 81 women and 9 men in each group. Ten out of 90 patients (11%) and 4 controls (4.4%) were TTV positive. Although the frequency of TTV positivity in patients is more than controls, there was no significant association between TTV and autoimmune hepatitis (p>0.05). There was no special pattern of disease in patients who presented TTV in their serum. Also, there was no association between TTV positivity and disease course, presence of autoantibodies, sex and age distribution.

It is known that autoimmune hepatitis results from a complex interaction between triggering factors, auto antigens, genetic susceptibility and immunoregulatory system. It has been speculated that liver cell destruction possibly caused by viral infection or toxic exposure might initiate the autoimmune process in susceptible individuals with genetic predisposition. It seems that a self-perpetuating autoimmune response may not necessarily require persistent infection with the virus related to the onset of autoimmune hepatitis. The possible pathogenesis is that various triggers may share epitopes that resemble self-antigens and break self-tolerance by overcoming antigenic ignorance. Molecular mimicry between foreign antigens and self-antigens is the most frequently proposed initiating mechanism.

There is no data available regarding the prevalence of TTV in our region, and the involvement of this virus in the pathogenesis of autoimmune hepatitis remains unclear. Accumulating data have demonstrated a high prevalence of this virus even in general population, TTV DNA has been detected in approximately 2% of blood donors in the United Kingdom, 1-10% in the United States of America, 10% in Columbia, 11% in Spain, 13% in Germany, 14% in Korea, 12-40% in Japan and 62% in Brazil. However, no pathology has yet been associated unequivocally with this virus. In addition, the initial observation of its hepatotropism has not been confirmed, and
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even injection of the virus into chimpanzees, while capable of causing infection, did not produce clinical illness.\textsuperscript{7,8} Thus, it remains elusive whether the virus was the cause of the liver disease, or was an incidental finding in initial studies.

In our study, although the frequency of TTV DNA in patients with autoimmune hepatitis was more than controls; we did not find any significant association between them. Furthermore, there was no relationship between the presence of TTV and the disease course, presence of autoantibodies, age and sex distributions, which suggest that there is no relation between TTV and autoimmune hepatitis at least in our study population, however, we suggest more studies with large number of study population and long term follow up before and after the onset of disease to be able to evaluate the exact role of TTV in auto immune hepatitis and other disorders.

References


