Tumor necrosis factor-alpha gene promoter polymorphism in Iranian patients with chronic hepatitis B


Research Center of Gastroenterology and Liver Disease (RCGLD), Taleghanie Hospital, 7th floor, Tabnak Street, Evin, Tehran, Iran

**Background:** Clearance of hepatitis B virus (HBV) infection requires a good host immune response. Cytokines like tumor necrosis factor-alpha (TNF-α) may play a role in such immune response. Genetic changes in TNF-α gene promoter region are known to influence TNF-α expression. We therefore studied the role of one such mutation in chronic HBV infection.

**Methods:** Presence of -308 G/A polymorphism in the promoter region of TNF-α gene was looked for in 100 patients with chronic HBV infection, 91 subjects with spontaneously recovered HBV infection, and 89 healthy controls, using a PCR-RFLP method.

**Results:** Variant alleles (A/A or A/G) were found in 22 of 100 (22%) patients with chronic HBV infection, 21 of 91 (23%) subjects with spontaneous HBV clearance, and 14 of 89 (15.7%) control subjects (p=ns for inter-group comparisons).

**Conclusion:** TNF-α promoter polymorphism -308A is common in Iranian population, but has no association with development of chronic HBV infection. [Indian J Gastroenterol 2006;25:14-15]

Several environmental, pathogen and host genetic factors determine the outcome of hepatitis B virus (HBV) infection. Of these, age at the time of acquisition of infection is the most important, with 90% of infants infected at birth, 25%-50% of children infected at age 1-5 years, and fewer than 5% of those infected during adult life developing chronic infection.

Tumor necrosis factor-alpha (TNF-α) is an important cytokine that determines host immune response to HBV and viral clearance. Persons with acute hepatitis B have been shown to have higher plasma levels of TNF-α than controls. Amount of cytokine production is known to be influenced by cytokine gene polymorphisms, and such polymorphisms have been shown to be associated with certain diseases. We looked at the relationship of one such polymorphism with chronic HBV infection.

**Methods**
The study included 100 consecutive patients positive for HBsAg, including all of the patients referred to our hospital during 2001-2003, with history of impaired liver function test (transaminases over two times the upper limit of normal for at least a 6-month period), detectable IgG anti-HBc and/or sonographic and clinical findings compatible with chronic liver disease.

The second group included 91 subjects who had no clinical or laboratory evidence of chronic liver disease, but were positive for anti-HBs and total anti-HBc antibodies and negative for HBsAg. None had history of excessive alcohol consumption or intravenous drug abuse, and all were negative for anti-HCV and anti-HIV. Patients with another cause of liver disease (Wilson’s disease, hemochromatosis, α-1 antitrypsin deficiency) or history of receiving immunosuppressive or anti-viral drugs were excluded.

Eighty-nine health-care workers and healthy blood donors, who were negative for both HBsAg and anti-HBc, were studied as control subjects. They were matched to the chronic HBV cases for age and gender.

A blood specimen (2 mL) was obtained from each participant and DNA was extracted from buffy coat using the salting out method. A region of TNF-α gene containing nucleotide at position -308 was amplified using PCR with previously-described primer sequences (forward: 5’- AAA TGG AGG CAA TAG GTT TCC AGG GGC ATG –3’ and reverse: 5’-ACT TCT CAC ACT TCC TCA CCT CCC GGC TC –3’); the resulting PCR product was digested with BstXI restriction enzyme and analyzed using agarose gel electrophoresis. The patterns expected after digestion were as follows: allele G/G – 28 and 103 basepair (bp) bands; allele G/A – 28, 103 and 131 bp bands, and A/A – 131 bp. Data were analyzed using chi-squared test (SPSS for Windows software, version 11.5); p value below 0.05 was considered significant.

**Results**
Mean (SD) age of patients with chronic HBV infec-
Table: Frequency of various TNF-α -308 alleles in subjects with chronic hepatitis B, self-limited HBV infection and healthy controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Chronic HBV infection (n=100)</th>
<th>Self-limited HBV infection (n=91)</th>
<th>Healthy controls (n=89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (G/G)</td>
<td>78 (78)</td>
<td>70 (77)</td>
<td>75 (84)</td>
</tr>
<tr>
<td>Heterozygous variant (G/A)</td>
<td>20 (20)</td>
<td>20 (22)</td>
<td>20 (22)</td>
</tr>
<tr>
<td>Homozygous variant (A/A)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Data as number (%); p=ns for intergroup comparisons

Discussion
Replacement of nucleotide G in TNF-α gene at -308 location with A has been shown to be associated with elevated TNF-α transcriptional activity and higher circulating levels of TNF-α.

TNF-α is a principal mediator of inflammation and cellular immune response, regulating at both transcriptional and post-transcriptional levels, and has been suggested to have an important role in noncytolytic and cytolytic clearance of HBV. Thus, in a previous study, spontaneous HBV clearance was associated with the presence of TNF-α -308A allele. However, another study failed to find such an association. Our study had results similar to those of the latter study. Our failure to find an association may be related to a type II error, and larger studies may prove more helpful.

The overall frequency of allele -308A in TNF-α gene promoter was 0.22 in the present study. This figure is higher than that reported in populations from other regions, viz., Taiwan (0.14), Sweden (0.20), Gambia (0.16), Caucasians in the US (0.14) and Japan (0.017). Thus, this polymorphism may be more common in Iranians. Ethnic differences in the distribution of cytokine gene polymorphisms have been described previously, and such variation could account for the higher frequency in our population.

References

Correspondence to: Dr. Somi. Fax: +98 (411) 336 7499. E-mail: mhosseinsina@yahoo.com

Acknowledgements: We acknowledge the study participants and their families, and our coworkers in RCGLD and GI ward in Talegani Hospital

Received June 22, 2005. Received in final revised form September 29, 2005. Accepted October 17, 2005