Association of TNF-α Promoter Polymorphism with HBV Associated Disease Outcome Among HBV Infected Patients from Orissa, Southern Part of East India

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Background: ***TNF-α*** **promoter polymorphism has been known to be a potential predictive factor in patients with HBV infection. We therefore tried to investigate whether the** ***TNF-α*** **promoter polymorphism at position −238, −857 and −863 was associated with the outcome of HBV infection in a population from Orissa, southern part of East India.** ***Methods:*** **A total of 195 patients recruited for the study were classified into 85 controls and 110 HBV infected cases, which included 34 IC, 30 CLD, 32 LC and 14 HCC patients. The polymorphisms at the respective sites were detected by a PCR-RFLP followed by statistical analysis.** ***Results:*** **The frequency of the genotype −238 GG and the allele −238G in the cases (89.0% and 92.7% respectively) was significantly higher than that in the controls (68.2% and 82.2% respectively) (*P* < 0.001, OR = 3.8 and *P* = 0.001, OR = 2.73). Whereas the −238 GA genotype was significantly high in the control group (28.2%) when compared to the cases (7.2%) (*P* < 0.001, OR = 0.2). Similarly, the frequency of −863CC and the allele −863C was significantly higher among the cases (24.5% and 49.5%) compared to controls (1.17% and 34.7%), (*P* < 0.001, OR = 27.32 and *P* = 0.003, OR = 1.85), whereas the −863CA genotype was significantly high in the controls (67.0%) when compared to the cases (50.0%) (*P* = 0.01, OR = 0.49). Haplotype −863C/−857C/−238G in cases was significantly higher than controls (*P* = 0.002). Multivariate logistic regression analysis indicates that the genotype −863CC bears a negative association with liver disease progression.** ***Conclusion:*** **The present study established an association of polymorphisms at site −238 and −863 of the TNF-α promoter with the outcome HBV infection and disease progression. (J Clin Exp Hepatol 2014;4:202–208)**

HBV Infection

Hepatitis B virus (HBV) infection in adults is usually clinically unapparent, and the virus is cleared after infection. Only about 5–10% of adults become persistently infected and develops chronic liver disease with varied severity,[1] which could not be explained completely by the virus itself or the environmental factors. Progress of HBV infection might be affected by host genetic susceptibility.[2] Due to the non-cytolytic nature of HBV, the hepatocellular injuries caused by HBV infection are principally immune mediated.[3–6] Host Immune attacks against HBV are mainly facilitated by a cellular reaction. Cytokines play an important role in defense against viral infection, indirectly through determination of the predominant pattern of the host response, and directly through inhibition of viral replication.[7] With the implication of host immune response against HBV and increase in viral pathogenesis, several researchers presently pay more attention to special clinical manifestation in relation to alleles responsible for antigen-presenting system and cytokines, that show polymorphisms and can also influence susceptibilities and outcomes of HBV infection.

Tumor necrosis factor-alpha (TNF-α) is produced by macrophages, monocytes, neutrophils, T-cells and NK-cells. It is synthesized as a membrane protein, which is cleaved to produce its soluble 17 Kd form. TNF-α, a principal mediator of cellular immune response and inflammation can stimulate cytokine secretion, increase the expression of adhesion molecules as well as activate neutrophils. There are several reports pertaining to the role TNF-alpha in HBV infection. It might have an important function in non-cytolytic and cytolytic clearance of

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Abbreviations: CLD: chronic liver disease; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; IC: inactive carrier; LC: liver cirrhosis; TNF-α: tumor necrosis factor alpha
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HBV.[8–10] Reports indicate that an elevated serum TNF-α level in chronic HBV-infected patients before the seroconversion of HBeAg was associated with fatal outcome in hepatic failure.[8,11] Moreover, the expression of human leukocyte antigen (HLA)-II molecules is also affected by TNF-α, and viral antigen presentation.[12]

Human TNF-α gene is known to be located within the class-III region of HLA in chromosome 6, and is closely associated to HLA gene clusters. It is well known that HLA polymorphisms are related with HBV infection outcomes.[13] Therefore, the TNF-α gene is vindicated as a candidate gene in the study of genetic susceptibility in HBV infection. There are many SNPs within the TNF gene which can influence the capacity of cytokine production in an individual. In the promoter, these are at positions (relative to the transcription start site) −1031 (T → C), −863 (C → A), −857 (C → A), −851 (C → T), −419 (G → C), −376 (G → A), −308 (G → A), −238 (G → A), −162 (G → A), and −49 (G → A).[14]

Literature studies on various diseases have shown that specific TNF genotypes resulting from these SNPs in the TNF-α promoter were associated with high or low levels of TNF-α secretion,[15–23] but ethnic difference could lead to different results. This association has earlier been investigated in various case control studies across the world, yet such reports from India are still scanty. Earlier two independent studies, one from North India[24] and the other from south India[25] have found that TNF polymorphisms were associated with the outcome of HBV infection. Therefore, the aim of the present study was to investigate whether the TNF-α promoter polymorphism at position −238, −857 and −863 was associated with the clinical outcome in an HBV infected population of Orissa, the southern part of East India.

MATERIALS AND METHODS

Study Subjects

A total of 195 samples enrolled in this study were collected from Berhampur Medical College, Berhampur and Sriram Chandra Bhanj (SCB) Medical College & Hospital, Cuttack, Orissa, India. Subjects were divided into two different groups according to serologic tests, HBV virological index, liver functional indexes and symptoms of hepatitis B infection, 85 controls (82 male and 3 female) and 110 cases (98 male and 12 female). The cases were further divided into inactive carrier (IC, n = 34), Chronic liver disease (CLD, n = 30), Liver cirrhosis (LC, n = 32), and hepatocellular carcinoma (HCC, n = 14). The criteria selected for grouping subjects into 'controls' and 'cases' were as follows. The control subjects were definitely negative for hepatitis B surface antigen (HBsAg) and HBV DNA, but positive for anti-HBc antibody, had normal liver functional tests. Subjects who served as cases were defined as explained before.[26] All HBV infected patient recruited for the study harbored HBV/D genotype. We excluded subjects who were positive for anti-HCV or anti-HIV. Patients with any other types of liver disease such as autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis or Budd-Chiari Syndrome and NASH were also excluded. Signed informed consents were obtained from all subjects recruited in the study.

Serological Testing

Commercial ELISA kits were used for HBsAg [Biomerieux, Boxtel, The Netherlands], anti-HCV [Ortho-Clinical Diagnostics, Raritan, N.J., USA] and anti-HIV [Biomerieux] detection.

Genotyping

Genomic DNA was extracted from blood clots using QIAamp genomic DNA kits (Qiagen, Netherlands) following the manufacturer's instructions. The SNPs at the positions −238(G/A) (rs361525), −857(C/T) (rs1799724) and −863(A/C) (rs1800630) (Table 1) were detected by Polymerase Chain Reaction–Restriction fragment length polymorphism (PCR-RFLP).[20,21,27] In brief a 152-bp, a 131-bp and a 133-bp fragment were amplified by PCR for positions −238 G/A, −857C/T and −863 A/C respectively. PCR was performed in a 25 μl reaction using 0.3 units of Chromous Taq polymerase (London, UK), 0.2 pmol of forward/reverse primers and 50 μg of DNA in a Perkin Elmer thermocycler (2720, Applied Biosystems). The PCR products of −238G/A [GG: 132 + 20 bp, GA: 152 + 132 + 20 bp, AA: 152 bp], −857C/T [CC: 106 + 25 bp, CT: 131 + 106 + 25 bp, TT: 131 bp] and −863C/A [CC: 108 + 25 bp, CA: 133 + 108 + 25 bp, AA: 133 bp] region were digested overnight at 37 °C with MspI, HincII and StyI restriction enzymes respectively. The digestion products were separated on 3% agarose gel and visualized directly under UV light with ethidium bromide staining. Genotyping of around 50% of the samples, chosen randomly were repeated in order to confirm the results. Viral genotyping was done as discussed earlier.[28]

Statistical Analysis

The Epi Info v 3.5.0 (Centers for Disease Control & Prevention, USA and World Health Organization, Geneva,

Table 1 Selected Single Nucleotide Polymorphisms Located at the Promoter Area of the Tumor Necrosis Factor-α Gene.

rs number	Gene	Location	Substitution	Minor allele
rs1800630	TNF-α	Promoter (−863)	A/C	A
rs1799724	TNF-α	Promoter (−857)	C/T	T
rs361525	TNF-α	Promoter (−238)	A/G	A

Table showing the SNPs included in the study, their position in the TNF-α promoter and the polymorphism type.