Gene 568 (2015) 140–145

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

The relationship between TNF alpha gene polymorphisms (−238/−308), TNF RII VNTR (p75) and outcomes of hepatitis B virus infection in Tunisian population

Ikram Sghaier [a,*], Sabrina Zidi [a], Leila Mouelhi [b], Radhouane Dabbech [b], Ezzedine Ghazouani [c], Etienne Brochot [d,e], Mouna Stayoussef [f], Besma Yacoubi-Loueslati [a]

[a] University de Tunis El Manar, Faculty des Sciences de Tunis, LR 206 Micro-organisms and Bio-molecules Actives, Tunisia
[b] Charles Nicolle Hospital, Hepato-Gastroenterology Department, Tunis, Tunisia
[c] Military Hospital of Tunis, Laboratory of Immunology, Tunis, Tunisia
[d] Department of Virology, Amiens University Hospital, Amiens, France
[e] Virology Research Unit, EA 4294, Jules Verne University of Picardie, Amiens, France
[f] Research Unit of Haematological and Autoimmune Diseases, Faculty of Pharmacy, University of Monastir, Monastir, Tunisia

ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form 11 May 2015
Accepted 12 May 2015
Available online 14 May 2015

Keywords:
TNF-α
Hepatitis B Virus
Gene polymorphism
HCC
TNF RII (p75)
Gene polymorphism
Cirrhosis

ABSTRACT

The present study was undertaken to investigate the association between Hepatitis B Virus (HBV) infection and polymorphisms of tumour necrosis factor alpha TNF-α −308 G>A, TNF-α −238 G>A and TNF RII VNTR (p75) gene promoter in a Tunisian population. Blood samples were collected from 100 Tunisian patients with HBV infection, 45 with Chronic Hepatitis (CH), 36 with Liver Cirrhosis (LC), 15 with Hepatocellular Carcinoma (HCC) and 200 healthy individuals of similar ethnicity. Genomic DNA was extracted from peripheral blood leukocytes. Genotyping of the analysed polymorphisms was performed using Amplified Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR), Restriction Fragment Length Polymorphism (RFLP) and Variable Number Tandem Repeat PCR (PCR-VNTR). The variant homozygotes −308 GG were associated with 50% decreased risk of HBV chronic infection (GG vs AA + GA; $p = 0.010$; OR = 0.50; 95%CI = 0.29–0.85). However, the carriers of minor allele −308 A have higher risk (1.5 times) to develop a chronic infection than other patients ($p = 0.027$; OR = 1.46; 95%CI = 1.04–2.06). The minor allele of −238 polymorphism was positively associated with virus resistance and the development of chronic infection ($p = 0.043$; OR = 1.42; 95%CI = 1.01 1.99). The distribution of −308, −238 and TNF RII VNTR (p75) among the three groups differed significantly. For HCC groups, there were statistically significant differences in allele distribution in −308, −238 respectively in which A allele remains a risk factor for HBV evolution to HCC ($p = 0.008$ and $p = 0.026$). Haplotype analysis revealed that TNF-α (−308A; −238A) was significantly associated to HBV chronic infection and moreover to disease aggravation to HCC stage. Our findings imply that variations in the genes governing the levels of constitutive and inducible TNF-α and TNF RII might be an important risk factor, which could explain the variable outcomes of HBV infection.

1. Introduction

Hepatitis B virus infection is a global health problem, with more than 2 billion people worldwide infected. Tunisia is among the countries of intermediate endemicity for hepatitis B, with current infection rates from 4% to 7% in the general population; hepatocellular carcinoma (HCC) representing 1.1% of all cancer deaths (Tunisian census, 2014). The most important result of HBV infection is the progression to HCC. In fact, three step process involving firstly chronic active hepatitis B, leading to the second step of cirrhosis and finally HCC which is the most common type of liver cancer (Lee William, 1997).

These different infection outcomes were mainly determined by virological and host factors (Wang, 2003). In recent years, the role of host immunological and genetic factors on hepatitis B pathogenesis has raised many questions. Cytokines play an important role in the host defence against viral infection because they indirectly determine the predominant pattern of the host response which can directly inhibit viral replication (Koziel, 1999). Among antiviral cytokines, TNF-α plays

Abbreviations: HBV, hepatitis B virus; TNF, tumour necrosis factor; HCC, hepato cellular carcinoma; ESLD, end stage liver disease; LC, liver cirrhosis; PCR, polymerase chain reaction; ARMS-PCR, Amplified Refractory Mutation System-Polymerase Chain Reaction; PCR-RFLP, Restriction Fragment Length Polymorphism; PCR-VNTR, Variable Number Tandem repeat; SNPs, single nucleotide polymorphisms; RT-PCR, Real Time Polymerase Chain Reaction; HCV, hepatitis C virus; d NTP, deoxyribo-nucleotide-tri-phosphate; OR, odds ratio; DNA, deoxyribonucleic acid; EDTA, ethylen-ediamine-tetra-acetic.

* Corresponding author at: Faculty des Sciences de Tunis, LR 206 Micro-organisms and Bio-molecules Actives, 1092 Tunis, Tunisia.
E-mail address: sghaiere.ikram@gmail.com (I. Sghaier).

http://dx.doi.org/10.1016/j.gene.2015.05.029
0378-1119/

a pivotal role in host immune response against HBV infection. Recently, Hrong-tay Tzeng et al. (2014) has shown that HBV persists in the absence of tumour necrosis factor alpha. In fact, it mediates the innate immune response leading to HBV clearance and to the blockage of HBV reactivation mechanism. The circulating TNF-α level increases during HBV infection (Tzeng et al., 2014; Schwabe and Brenner, 2006) which is correlated with the severity of hepatic inflammation, fibrosis, and tissue injury (Akpolat et al., 2005; Falasca et al., 2006). In fact, over-expression or systemic exposure to TNF-α cytokine may be harmful leading to initiation of the process of fibrosis, cirrhosis, and, eventually HCC (Cha and De Matteo, 2005; Jen-Eing et al., 2007). HCC is a heterogeneous tumour with a complex variety of genetic changes. It is clear that chronic hepatitis B is a common precursor of cirrhosis and HCC since it induces hepatocyte proliferation and genetic alteration (Cha and De Matteo, 2005).

The TNF-α exerts its biologic activity by interacting with two distinct TNF receptors, with molecular masses of 55 kD (TNF RI) and 75 kD (TNF RII), respectively (Bazzoni and Beutler, 1996; Allendoerfer et al., 1993; Steinshamn et al., 1996). Various studies have shown that after an appropriate stimulation, both TNF receptors may be shed from the cell surface of many cell types, resulting in the expression of soluble forms of these proteins that retain binding affinities for TNF-α. Although the role of TNF-Rs remains a subject of speculation, soluble forms of each receptor inhibit TNF-α action, and can down regulate the inflammatory response (Leeuwenberg et al., 1994; Pinckard et al., 1997).

Data from several systems strongly suggest that a bi-allelic variable number tandem repeat (VNTR) in the promoter of the TNF RII gene, which involves the insertion/deletion of a 15 bp sequence, has a role in signal transduction during pathogenesis (Santee and Owen-Schaub, 1996) thus it can play an active role in elicitation of cytokine secretion (Vandenabeele et al., 1992). In fact, a study of Grell et al. (1995) indicates that the transmembrane form of TNF-α is the prime activating ligand of TNF RII and it can give qualitatively different results compared with soluble TNF-α (Grell et al., 1995). Thus, tumour cells that are resistant to soluble TNF-α can be made sensitive by activating TNF RII with the transmembrane form of TNF-α. The latter, indicates that TNF RII activation may be more important in localized inflammatory responses. To the best of our knowledge, no studies have yet analysed the association between the polymorphism of this receptor and the susceptibility to HBV infection.

Several studies have suggested that cytokine gene polymorphisms, especially those polymorphisms within the regulatory regions or signal sequences of cytokine genes, can affect the different outcomes of HBV infection leading to cirrhosis or/and HCC (Ben-Ari et al., 2003; Qi et al., 2011). Some of these results were conflicting. Particularly, for two bi-allelic single nucleotide polymorphisms (SNPs) at the −238 (rs361525) and −308 (rs1800629) positions: two G versus A transitions in the promoter region had been shown to influence TNF-α expression. In fact, the A −238 allele was associated with down regulation of TNF-α production (Huizinga et al., 1997; Hajeer and Hutchinson, 2000; Bayley et al., 2001; Scardapane et al., 2012) while the A −308 allele was associated with higher constitutive levels of TNF-α (Wilson et al., 1997). Many studies had investigated the association of these two polymorphisms with the susceptibility to HBV infection. The results were inconsistent (Cheong et al., 2006; Wang et al., 2010), in fact, some studies had shown that patients with AA genotype for −308 and −238 have been at higher risk of susceptibility of persistence of HBV (Chen et al., 2010; Kim et al., 2003; Niro et al., 2005) while other studies had shown contradictory results (Cheong et al., 2006; Ribeiro et al., 2007; Somi et al., 2005).

Until now, the effect of TNF-α gene −238 and −308 and the TNF RII (insertion/deletion of 15 bp) polymorphisms in susceptibility to HBV infection and/or its outcomes has not yet been analysed in Tunisians. In this context, we aimed to investigate whether these polymorphisms have an impact on clearance of HBV and outcome of HBV chronic hepatitis.

2. Materials and methods

2.1. Subjects: cases and controls

In a prospective study from October 2012 to December 2013, 100 Tunisian cases with hepatitis B confirmed by detection of viral HBV level using RT-PCR were consecutively recruited from Charles Nicolle Hospital. Patients were classified according to their infection status and the severity of their disease using stringent clinical and laboratory criteria into three groups: group 1 (G1) consisting of 49 patients with chronic infection of hepatitis B; group 2 (G2) consisting of 36 subjects with liver cirrhosis (LC) and group 3 (G3) regrouping 15 cases with HCC. Data from each patient including gender, age at diagnosis, possible risk factors for HBV such as transfusion, diabetes, auto-immune disease, co-infection with HCV, dental care, sexual relation, surgical operations, tobacco consumption, alcohol consumption and anti-viral treatment were obtained by questionnaire, personal interviews and reviews of case records (Table 1). The control group was composed of 200 ethnically and geographically matched healthy individuals, with evidence of negative tests for HCV antibodies and free from any chronic clinical problem and disease manifestation; no history of malignancy, drug allergy, hypertension, diabetes or cardiovascular disease. The serological status of every subject with regard to HBsAg, anti-HBs, and antiHBc (IgG and IgM), were determined by ELISA. Written informed consent was taken from all study subjects prior to study enrolment and the protocol was approved by the local ethics committee of Charles Nicolle Hospital of Tunis.

2.2. Blood collection and DNA extraction

Five millilitres of venous blood with EDTA, as anticoagulant, was collected from each subject. Genomic DNA was extracted using QIAamp DNA blood Mini Kit (Qiagen GmbH, Hilden).

2.3. HBV DNA detection

HBV DNA was determined by a commercially available molecule hybridization assay (COBAS Taq Man HBV test — Roche Diagnostics).

2.4. TNF-α genotyping

Polymorphisms at positions −238 (rs361525), −308 (rs1800629) of TNFα gene and bi-allelic VNTR (p75) (rs5745546) of TNF RII gene were genotyped. The G and A alleles at position −238 were identified by PCR-RFLP, using the following primers: Forward 5′AGAAGACCCCC CTCGGAACC3′ and Reverse 5′ATCTGGAGGAAGCGGTAGTG3′, as previously described (Li et al., 2006). The G and A alleles at position −308 in the promoter region of the TNF-α gene were identified using ARMS-PCR methodology (Perrey et al., 1999). Primer sequences were as follows: generic primer: 5′TCACGGATTTCTGTTGTGTTTC3′, primer specific for G allele: 5′ATAGGTTTTGAGGGGCATGG3′ and primer specific for A allele: 5′AATAGGTTTTGAGGGGCATGA3′. The amplification of the −238 and −308 was performed in a volume of 25 μL containing 50 ng of genomic DNA, 20 pmol/L of each primer, 200 μmol/L dNTP, 1.5 mmol/L $MgCl_2$, buffer and 1 U Taq polymerase. PCR procedure of TNF −238 was as follows: pre-denaturation at 95 °C for 5 min, 30 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR products were digested with Msp I restriction enzyme. TNF-α −238 allele G was identified by 20- and 132-bp fragments and allele A by a single 152 bp fragment. However, the PCR procedure of TNF −308 amplification region was: pre-denaturation at 95 °C for 10 min, 30 cycles of denaturing at 95 °C for 1 min, annealing at 59 °C for 50 s, extension at 72 °C for 50 s, and a final extension at 72 °C for 10 min. The genotyping of the VNTR (p75) (rs5745946) in the promoter of the TNF RII gene, which involves the insertion/deletion of 15 bp fragment, was