

Stromal Cell-Derived Factor-1 chemokine genetic polymorphism in hepatitis B patients

Polimorfismo genético da quimiocina Fator Derivado do Estroma da medula óssea em pacientes com hepatite B

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Abstract

Hepatitis B virus (HBV) is noncytopathic, hepatotropic and cause acute and chronic necroinflammatory liver diseases. Many chemokine and yours receptors are involved in the HBV pathogenesis, with a role at the infection capacity and the host response. Sequence analysis of a common variant revealed a G→A transition at position 801 (counting from the ATG start codon) in the 3' untranslated region (3' UTR) of the reference sequence (GenBank L36033) consist the polymorphism designated SDF1-3'UTR-801 G→A and abbreviated SDF1-3'A which is represented in the SDF-1 β but not in the SDF-1 α transcript. The objective of this study was to evaluate the genotype of patients infected with HBV through a PCR-RFLP. The present study focuses the same distribution of the SDF-1 allele in the HBV positive and negative individuals.

Keywords: SDF-1 β , HBV, polymorphism

Resumo

O vírus da hepatite B (HBV) é um vírus não citopático, hepatotrópico e provoca doenças hepáticas inflamatórias agudas e crônicas. Muitas citocinas e seus receptores estão envolvidos na patogênese do HBV, com papel na capacidade de infecção e na resposta do hospedeiro. A análise da sequência de uma variante comum revelou uma transição G→A na posição 801 (contando a partir do códon de início ATG) na região 3' não traduzida (3' UTR) na sequência de referência (GenBank L36033) consistindo em um polimorfismo designado SDF1-3'UTR-801 G→A e abreviado como SDF1-3'A, o qual é representado no transcrito SDF-1 β mas não no SDF-1 α . O objetivo deste trabalho foi avaliar o genótipo dos pacientes infectados por HBV, através de uma PCR-RFLP. O presente estudo verificou distribuição semelhante do alelo SDF-1 em pacientes HBV positivos e indivíduos saudáveis.

Palavras-chave: SDF-1 β , HBV, polimorfismo.

INTRODUCTION

Hepatitis B virus (HBV) is hepatotropic member of the hepadnavirus family that is spread by contact with infected blood and body fluids and cause acute and chronic necroinflammatory liver diseases ⁽¹⁻⁵⁾.

HBV infection in immunocompetent adults results in a self-limited, transient liver disease and viral clearance in more than 95% of adults, whereas more than 90% of neonates exposed to HBV at birth become persistently infected. Persistent HBV infection is associated with varying degrees of chronic liver disease, and it often progresses to the development of cirrhosis and hepatocellular carcinoma (HCC) ^(1,2). Chronic hepatitis B infection is frequently diagnosed within the genitourinary clinic setting with sexual transmission the commonest route of acquisition.

Only 3-5% of adults who contract acute hepatitis B will progress to chronic infection, and these individuals can be identified by the presence of hepatitis B surface antigen (HBsAg) in the bloodstream 6 months after infection. Individuals at highest risk of long-term complications such as cirrhosis and hepatocellular carcinoma carry HBeAg and have high levels of circulating hepatitis B virus (HBV) deoxyribonucleic acid (DNA)⁽⁴⁾. More than 350 million people are chronically infected by HBV, and approximately one million die from these late complications each year worldwide ⁽¹⁻³⁾.

It is well established that the function of some molecules on the surface of leukocytes is critically regulated by activating events triggered by chemoattractants binding to specific receptors on the leukocytes. These activation events allow them to stick firmly to and, eventually, to emigrate through the vessel wall into the extravascular sites ⁽⁵⁾.

Hepatitis B virus (HBV) infection is accompanied by inflammation and fibrosis eventually leading to cirrhosis. The chemokine SDF (named: CXCL12) is involved in chronic inflammatory conditions. The role of the chemokine CXCL12 and its receptor CXCR4 pathway in HBV-associated liver inflammation and fibrosis was therefore studied ⁽⁶⁾. Wald et al (2004) found that CXCL12 is expressed by bile duct epithelial cells in normal liver tissue. Bile duct proliferation and liver fibrosis in chronic HBV infection result in the anatomical re-distribution of CXCL12 in the liver. Moreover, CXCL12 is up-regulated in the endothelium of neo-blood-vessels formed in active inflammatory foci and is significantly elevated, compared with controls, in the plasma of patients with advanced liver fibrosis. Complementing these observations were others indicating that over 50% of liver-infiltrating lymphocytes express CXCR4 and, in response to CXCL12, migrated and adhered to fibronectin. These observations suggest an important role for the CXCL12/CXCR4 pathway in recruitment and retention of immune cells in the liver during chronic HBV infection.

A polymorphism in an evolutionary conserved segment of the 3' untranslated region (3'UTR) of the SDF-1 structural gene transcript (SDF-3'A) was described. Persons homozygous for this mutation had a significantly delayed progression to AIDS and were even more strongly protected from death. It was hypothesized that the SDF1-3'A mutation could result in increased SDF-1 production, resulting late in infection in strong competition with virus variants at the CXCR-4 receptor level ⁽⁷⁾.

The present study focuses on distribution of the SDF-1 allele in HBV Brazilian patients representative of the European, African and Asiatic ethical descendent.

SUBJECTS AND METHODS

Study population

We studied blood sample from 45 HBV patients. All the samples were taken from Regional North Paraná University Hospital from Londrina State University. We also studied 30 blood samples from healthy individuals.

Immunological assays

Serum samples were examined by Microelisa System for HbsAg and HBc (AxSYM).

SDF-1 genotyping

Genomic DNA was isolated from peripheral blood cells using “Super Quick-Gene DNA Isolation Kit” – Analytical Genetic Testing Center (AGTC) and 100 ng of DNA was analyzed by PCR with primers SDF 3’UTR-F (sense) and SDF 3’UTR-R2 (antisense). The G→A transition in SDF1-3’A alleles eliminates a MspI site allowing the use of a PCR restriction fragment length polymorphism assay for rapid detection of SDF-1 genotypes⁽⁷⁾. Samples were amplified with Taq polymerase (Pharmacia) in the buffer provided, with a final concentration of 1,5 mmol/l. Conditions of PCR comprised 5 min. denaturation at 94°C, 35 cycles of 1 min. at 94°C, 1 min. at 60°C and 1 min. at 72°C and 10 min. elongation at 72°C in a Perkin Elmer thermocycler. PCR products were subjected to restriction analysis with MspI for 3 h. at 37°C (Gibco, BRL, Maryland, USA) and analyzed on a 2% agarose gel, yielding a 101 and 193 base-pair product in the case of a SDF-1 wild-type allele (SDF1-wt) and a 294 base-pair product in the case of SDF1-3’A.

STATISTICAL ANALYZES

The Hardy-Weinberg equilibrium was determined through χ^2 test. The demographics characteristics were analyzed using the Microcal Origin™ 4.1.

RESULTS

Antibodies to the core of hepatitis B virus (anti-HBc) are considered to be the best serologically reliable markers of hepatitis B virus (HBV) infection. All positive samples to HBV used in this study presented anti-HBc reactivity and were negative to HbsAg and the group of patients and control were in Hardy-Weinberg equilibrium.

SDF-1 is the ligand to chemokine coreceptor (CXCR4) and from its three genotypes: wt/wt, 3’A/wt and 3’A/3’A (figure 1). Our study demonstrated the low frequency of SDF1-3’A/3’A, in 3 (6,67%) HBV patients and 2 (6,6%) from healthy individuals. The percentage of all three genotypes is presented in Table 1.

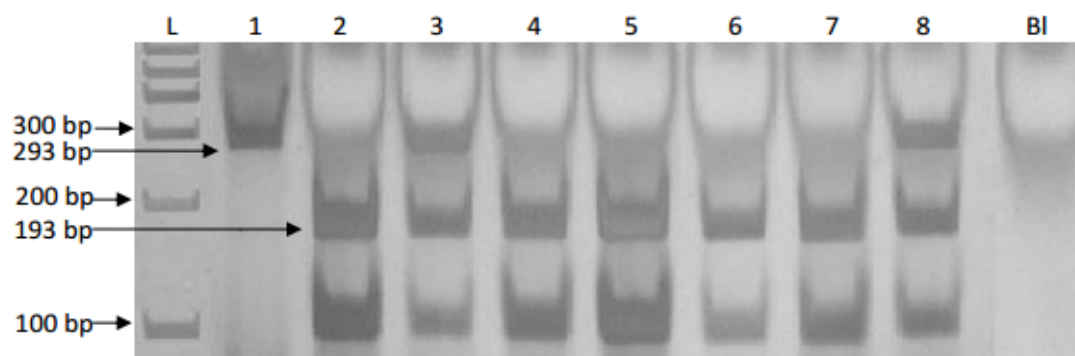


Figure 1. SDF-1 Genotyping. PCR products were subjected to enzymatic digestion by incubation with *Msp-I* (PROMEGA- USA) for 3h at 37°C and then submitted to electrophoresis in 2% agarose gels. Wild -type alleles (SDF1-wt) yielded 100 and 193 bp products, while SDF1-3'A alleles yielded a 293-bp product. L: Ladder; BI: Blank.

Table 1. SDF-1 chemokine gene variant in the HBV and normal donors

Samples*	wt/wt	3'A/wt	3'A/3'A
HBV (45)	30 (66,67%)	12 (26,67%)	3 (6,67%)
Normal donors (30)	20 (60%)	8 (33,33%)	2 (6,6%)

*Hardy-Weinberg Equilibrium: $1,25(\text{HBV patients}) \times 0,833(\text{normal donors})$ (1 degree of freedom; $p > 0,05$)

DISCUSSION

It has been known that viral tropism which is determined by the ability of the some virus to bind to specific chemokine coreceptor on the cells. There are many chemokine receptors used most commonly by pathogenic virus⁽⁸⁾ like CCR5 and CXCR4 for the HIV virus^(9,10). Stromal-derived factor (SDF-1, also named pre-B cell growth stimulating factor), a powerful chemoattractant cytokine, is the natural and exclusive ligand for CXCR4, was initially identified as a bone-marrow stromal cell-derived factor⁽¹¹⁾, and as a pre-B-cell stimulatory factor^(12,13). It is also known as highly efficient lymphocyte chemoattractant^(14,15).

The mobilization of hematopoietic stem cells from the bone marrow to the blood circulation guarantees a sufficient number of peripheral blood progenitor cells (PBPCs). The SDF-1 plays an important role in this process, leading to the normal development

of hematopoietic cells and influences normal development of the embryo⁽¹⁶⁾. SDF-1 also has an important role in the directional migration of cancer cells during the metastatic process⁽¹⁷⁾. Experiments have shown that SDF-1 β (one of two transcriptional splice variants of the SDF1 gene) is capable of down-regulating CXCR-4 on cells by endocytosis⁽¹⁸⁾.

In our study we detected lower frequency to 3'A/3'A in the HBV and normal donors. In the other hands, the wild type (wt/wt) was the most common genotype. The question to be elucidated is the possible correlation of delayed progression from HBV individuals and homozygous SDF genotype. Others studies also correlated some chemokine and chemokine receptors with HBV progression. CCR5 polymorphism delta 32 was evaluated, and was proposed that it reduced the risk of developing a persistent HBV infection^(19,20).

We verified presence of this homozygous genotype in the 3 (6,52%) HBV positive individuals and 2 (6,6%) healthy individuals. The present study demonstrated the similar distribution of the SDF-1 allele in the all individuals analyzed and therefore further studies should be conducted so that we can make an association between this polymorphism and hepatitis B.

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