Major Article

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The absence of the human platelet antigen polymorphism effect on fibrosis progression in human immunodeficiency virus-1/ hepatitis C virus coinfected patients

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ABSTRACT

Introduction: Hepatic fibrosis progression in patients with chronic hepatitis C virus infections has been associated with viral and host factors, including genetic polymorphisms. Human platelet antigen polymorphisms are associated with the rapid development of fibrosis in HCV-monoinfected patients. This study aimed to determine whether such an association exists in human immunodeficiency virus-1/hepatitis C virus-coinfected patients. **Methods:** Genomic deoxyribonucleic acid from 36 human immunodeficiency virus-1/hepatitis C virus-coinfected patients was genotyped to determine the presence of human platelet antigens-1, -3, or -5 polymorphisms. Fibrosis progression was evaluated using the Metavir scoring system, and the patients were assigned to two groups, namely, G1 that comprised patients with F1, portal fibrosis without septa, or F2, few septa (n = 23) and G2 that comprised patients with F3, numerous septa, or F4, cirrhosis (n = 13). Fisher's exact test was utilized to determine possible associations between the human platelet antigen polymorphisms and fibrosis progression. **Results:** There were no deviations from the Hardy-Weinberg equilibrium in the human platelet antigen systems evaluated. Statistically significant differences were not observed between G1 and G2 with respect to the distributions of the allelic and genotypic frequencies of the human platelet antigen systems. **Conclusions:** The greater stimulation of hepatic stellate cells by the human immunodeficiency virus and, consequently, the increased expression of transforming growth factor beta can offset the effect of human platelet antigen polymorphism on the progression of fibrosis in patients coinfected with the human immunodeficiency virus-1 and the hepatitis C virus.

Keywords: Coinfection. Hepatitis C virus. Human immunodeficiency virus. Human platelet antigen. Liver fibrosis.

INTRODUCTION

The progression of hepatic fibrosis in patients with chronic hepatitis C virus (HCV) infection depends on different viral and host factors. Sex, age at infection, consumption of alcohol, coinfection with the human immunodeficiency virus (HIV) and coinfection with the hepatitis B virus have been associated with liver fibrosis progression[1] [2] [3]. Similarly, host genetic factors have also been associated with this progression. Besides polymorphisms in the genes coding for human leukocyte antigens, other genetic polymorphisms in the genes coding for interleukin 10, tumor necrosis factor alpha, angiotensinogen, and transforming growth factor (TGF)-β1 have been associated with the progression of fibrosis in HCV-moinfected patients[4].

Similarly, the expression of some integrins, which are present in a variety of cell types, including platelets[5] and liver stellate cells[6] [7], has been associated with the development of fibrosis[8] [9]. The integrins that are present on platelet membranes express different types of antigens, and, of these, the human platelet antigens (HPA) have received considerable attention[10].

Human platelet antigens are polymorphic within the population, and the polymorphism in most of these antigens is caused by the substitution of a single amino acid in the protein, which is the consequence of the substitution of one nucleotide in the deoxyribonucleic acid (DNA)[11] [12]. The polymorphisms of some of the HPA systems have been associated with diseases involving platelets[13] and with disorders that do not involve platelet disturbances, including sickle cell anemia[14], myocardial

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infarction[15], and venous thrombosis[16]. Recently, HPA polymorphism has also been associated with viral infections, including dengue virus infections[17], HCV monoinfections[18], and HIV/HCV coinfection (Grotto et al: in press).

In addition, the findings from a recent study demonstrated an association between the HPA-1a/1b genotype and the rapid development of fibrosis in HCV-monoinfected patients[19]. However, whether associations exist between HPA polymorphisms and the progression of fibrosis in HIV/HCV-coinfected patients is unclear.

The aim of this study was to determine possible associations between HPA-1, HPA-3, and HPA-5 polymorphisms and the progression of hepatic fibrosis in individuals coinfected with HIV-1 and HCV.

METHODS

Patients

Samples of ethylenediaminetetraacetic acid-anticoagulated peripheral venous blood were collected from 36 HIV-1/HCV-coinfected patients who were seen at the Specialized Outpatient Service "Domingos Alves Meira" and at the Department of Internal Medicine, Gastroenterology Division, Botucatu School of Medicine, São Paulo State University, UNESP, Botucatu, SP, Brazil. The study's inclusion criteria were the presence of an HIV-1/HCV coinfection that was determined using the reverse transcription-polymerase chain reaction (RT-PCR) a liver biopsy performed before antiviral treatment began, the absence of other hepatic diseases, and the provision of signed informed consent.

The levels of fibrosis were determined from liver biopsies, which were obtained using Menghini or Tru-Cut needles, and the fragments were analyzed when at least eight portal spaces were present. The tissues underwent hematoxylin and eosin, Masson trichrome, and reticulin staining. The biopsies were analyzed by a pathologist who used the Metavir scoring system[20] to evaluate fibrosis progression. The patients were assigned to Group 1 (G1) that comprised HIV-1/HCV-coinfected patients with lower stages of fibrosis (F1, portal fibrosis without septa or F2, few septa) or Group 2 (G2) that comprised HIV-1/HCV-coinfected patients with higher degrees of fibrosis (F3, numerous septa or F4, cirrhosis). In the study, patients who were infected with the HCV genotypes 1, 2, or 3, and the HIV subtypes B, F, or BF recombinant were included. Information was obtained from the databank regarding each patient's sex, age, and time of infection, which was defined as the time that had elapsed between the presumed date of infection and the biopsy date; this information was available for 27 of the 36 patients studied. Information was also obtained about the degree of alcohol abuse, which was defined as more than 40g per day for women and over 80g per day for men; this information was available for 29 of the 36 patients studied.

Deoxyribonucleic acid extraction and human platelet antigen genotyping

Genomic DNA was isolated from whole blood using a commercial AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Scientific, Inc., Union City, CA, USA). HPA-1 and HPA-3 were genotyped using PCR sequence-specific primers according to Kluter et al.[21], and the *CRP* gene was used as the internal positive control in all reactions. HPA-5 was genotyped using PCR-restriction fragment length polymorphism analysis according to Kalb et al.[22]. A negative control was used in all of the PCR assays.

Statistical analysis

The Hardy-Weinberg equilibrium test was carried out to evaluate the distributions of the allelic frequencies of HPA-1, HPA-3, and HPA-5 according to the degrees of fibrosis. Fisher's exact test was used to investigate possible associations between the HPA alleles, genotypes, and the degrees of fibrosis in HIV-1/HCV-coinfected patients. The level of significance for all of the statistical tests was set at 0.05.

Ethical considerations

The study was approved by the Research Ethics Committee of Botucatu Medical School, UNESP (3750-2010). A written informed consent was obtained from patients participating in the study.

RESULTS

Table 1 shows the demographic and virological characteristics of the patients who participated in the study.

There were no deviations from the Hardy-Weinberg equilibrium in the HPA systems evaluated. No statistically significant differences were found between the patient groups in relation to the distributions of the allelic and genotypic frequencies of the HPA systems **(Table 2)**.

DISCUSSION

The progression of hepatic fibrosis in patients with HCV infections has been associated with factors that include sex, alcohol consumption, the age at infection[1][2][3], and genetic factors[4].

Fibrosis develops mainly as a consequence of the activity of the liver stellate cells[23][24]. The activation of the liver stellate cells has been associated with regulation by the integrin family of proteins[8][9], and some of these are expressed on hepatic stellate cells[6][7].

In 2012, Silva and coworkers[19] found an association between the genotype HPA-1a/1b and the more rapid progression of fibrosis in HCV-monoinfected patients, and the patients reached F3/F4 earlier. However, this was not observed among the HIV-1/HCV-coinfected patients in the present study, which suggests that the effect of HPA polymorphism on the progression of hepatic fibrosis that has been observed in HCV-monoinfected patients is lost in the presence of HIV.

Unlike the HCV, the HIV does not replicate in human hepatocytes. However, the chemokine coreceptors CCR5 and CXCR4, which allow the HIV to enter its target cells, are expressed on hepatocytes and stellate cells. The interactions between the HIV and these cells via CCR5 and CXCR4 induce cell signaling[25][26][27], which subsequently leads to the greater expression of pro-fibrogenic factors, such as TGF-β1, which

TABLE 1 - Demographic characteristics of the human immunodeficiency virus-1/hepatitis C virus-coinfected patients (n = 36) included in the study.

Characteristic	HIV/HCV-coinfected patients (n = 36)
Median age, years (IQR)	44.19 (38.8–49.3)
Men, n (%)	26 (72.2)
Mean duration of infection, years*	13.4
Abusive alcohol consumption, n (%)**	10 (27.8)
Fibrosis, n (%)	
G1 (F1–F2)	23 (63.9)
G2 (F3–F4)	13 (36.1)

HCV: hepatitis C virus; **HIV**: human immunodeficiency virus; **IQR**: interquartile range; **G1**: Group 1; **G2**: Group 2. *Twenty-seven samples evaluated. **Twenty-nine samples evaluated.

TABLE 2 - Allelic and genotypic frequencies of human platelet antigens 1, 3, and 5 in human immunodeficiency virus-1/hepatitis C virus-coinfected patients in Group 1 (F1 and F2) and Group2 (F3 and F4).

	G1 (n = 23)	G2 (n = 13)	p-value
Allele*			
1a	36	22	0.5129
1b	10	4	
3a	28	20	0.1652
3b	18	6	
5a	42	25	0.4369
5b	4	1	
Genotype*			
1a/1a	14	10	0.4296
1a/1b	8	2	
1b/1b	1	1	
3a/3a	8	8	0.4262
3a/3b	12	4	
3b/3b	3	1	
5a/5a	19	12	0.6336
5a/5b	4	1	
5b/5b	0	0	

F1 and F2: F1, portal fibrosis without septa or F2, few septa; **F3 and F4**: F3, numerous septa or F4, cirrhosis; **G1**: Group 1; **G2**: Group 2. *Analysis using Fisher's exact test.

are involved in the activation of the stellate cells. Thus, the presence of HIV-1 and the activation of signaling pathways via non-integrin receptors may lead to the greater stimulation of the stellate cells as a consequence of the increased expression of TGF-β, which would offset the effect of the HPA polymorphism on the progression of hepatic fibrosis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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