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Major Article

Do differences exist between chronic hepatitis C genotypes 2 and 3?

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ABSTRACT

Introduction: Six genotypes of the hepatitis C virus (HCV) have been identified thus far, and their distribution is well defined. Genotype 1, which is the most prevalent worldwide, is always compared to genotypes 2 and 3, particularly in terms of treatment response. However, little is known about the differences between genotypes 2 and 3 because these genotypes are analyzed together in most studies. Therefore, the aim of this study was to evaluate differences in the clinical, epidemiological, laboratory, and histological parameters between HCV-2 and HCV-3. **Methods:** Patients with chronic hepatitis C infected with genotypes 2 and 3 were studied retrospectively and compared according to clinical, laboratory, and histological aspects. Hepatitis C virus-ribonucleic acid (HCV-RNA) was analyzed quantitatively by TaqMan® real-time PCR, and the HCV genotype was determined by sequencing the 5'-untranslated region. **Results:** A total of 306 patients with chronic HCV-2 (n=50) and HCV-3 (n = 256) were studied. Subtype 2b (n=17/50) and subtype 3a (n=244/256) were the most prevalent among patients infected with HCV-2 and HCV-3, respectively. The mean age was 47 ± 10 years, and there was a predominance of men in the group studied (61%). Comparative analysis between HCV-2 and HCV-3 showed a younger age (p=0.002), less prevalence of arterial hypertension (p=0.03), higher serum albumin levels (p=0.01), more advanced stage of liver fibrosis (p=0.03), and higher frequency of steatosis in patients with HCV-3 (p=0.001). After multivariate regression analysis, all the variables, except serum albumin, remained as variables associated with HCV-3 in the final model. **Conclusions:** Clinical and histological differences exist between HCV-2 and HVC-3, which suggests the need for separate analyses of these genotypes.

Keywords: Hepatitis C. HCV-2. HCV-3. Fibrosis.

INTRODUCTION

Hepatitis C affects thousands of people around the world, causes chronic liver disease that can progress to liver cirrhosis, and is a leading cause of liver transplantation. The hepatitis C virus (HCV) is characterized by genetic heterogeneity, which permits its classification into distinct genotypes and subtypes by phylogenetic analysis[1]. This genetic diversity is related to the origin of the virus, its interesting evolution, its geographic and epidemiological pattern of dissemination, and its long persistence in humans[2,3]. Simmonds et al.[1] proposed the classification of HCV into six viral genotypes with several subtypes: 1 (1a, 1b, 1c), 2 (2a, 2b, 2c), 3 (3a, 3b), 4, 5 (5a), and 6. Genotypes 1a and 1b are the most prevalent in western countries[1], with genotype 1b being the most common in Europe[4-6] and genotype 1a being the most common in the United States[7,8]. Genotype 2 and its subtypes predominate in western Africa[9-11]. Genotype 3a is the second most common genotype in Europe after genotype 1[4,6]. Genotype 4 is prevalent in the Middle East[12-14] and in Central Africa[15-17], while genotype 5 is common in South Africa[1,18], and genotype 6 is found mainly in South Asia[19-21]. Genotype 7 has been found in Vietnamese patients with chronic hepatitis C, although some authors have advocated that it should be reclassified as a subtype of genotype 6[22].

In addition to the wide geographic distribution of HCV genotypes and subtypes, HCV exhibits a specific pattern of transmission. For example, genotypes 1a, 3a, and 4 are associated mainly with intravenous drug use, while genotypes 1b and 2 are associated with transmission through blood transfusion[23-25]. With respect to the treatment of chronic hepatitis C, genotype 1 is less responsive to antiviral therapy based on pegylated interferon and ribavirin[26]. In contrast, genotypes 2 and 3 show higher response rates to antiviral treatment, and their treatment duration is shorter compared to that of genotype 1[27].

In most studies, patients infected with genotype 1 are compared to those infected with genotypes 2 and 3, particularly in terms of treatment response. Moreover, little is known about the differences between genotypes 2 and 3 because these genotypes are usually analyzed together. The aim of the present

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study was to evaluate the distribution of HCV genotypes 2 and 3 and their subtypes as well as to determine differences or similarities in clinical, laboratory, and histopathological parameters between these two genotypes.

METHODS

Patients with chronic hepatitis C caused by genotypes 2 and 3 who were evaluated between 1994 and 2011 at the Hepatitis Unit of the Department of Gastroenterology, Federal University of São Paulo, were studied retrospectively. The criteria for inclusion in the study were as follows: age of 18 years or older, detection of HCV-RNA, and identification of HCV-2 and HCV-3. Patients who were co-infected with human immunodeficiency virus (HIV) or hepatitis B virus (HBV), organ transplant recipients, patients with end-stage renal disease, and patients with alcohol abuse were excluded from the study. Clinical, laboratory, and histological variables were compared between patients with HCV-2 and HCV-3.

Clinical and laboratory assessment

The following clinical variables were analyzed: gender, age, parenteral transmission risk factors (transfusion of blood and blood derivatives and/or intravenous drug use), estimated duration of infection in patients with a known parenteral transmission risk factor, body mass index (BMI), diabetes mellitus, and arterial hypertension. Laboratory tests included the measurement of serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (AP), bilirubin, albumin, cholesterol, triglycerides, glucose, platelet count, and prothrombin activity (PA).

Molecular tests

Qualitative HCV-RNA was detected by PCR using the Amplicor Hepatitis C Virus Test, version 2.0 (Roche Molecular Systems, Branchburg, NJ, USA) with a detection limit of 50IU/mL. HCV genotyping was performed by amplification followed by hybridization of the HCV 5'-untranslated region (INNo-LiPA HCV II, Innogenetics NV, Belgium)

Histological analysis

Histological analysis was used to evaluate the activity grade and fibrosis stage according to the METAVIR system. If no liver biopsy was available, the patients were classified as cirrhotic in the case of a suggestive radiological image (reduction in liver size, heterogeneous liver, and increased portal vein caliber) and/or evidence of portal hypertension based on the presence of esophageal varices upon digestive endoscopy. The presence of steatosis with or without steatohepatitis was analyzed, and steatohepatitis was classified according to the nonalcoholic fatty liver disease activity score[28].

Statistical analysis

Numerical variables are reported as means and standard deviations, and categorical variables are expressed as absolute and relative frequencies. Analyses of skewness and kurtosis and the Kolmogorov-Smirnov test were used to determine whether continuous variables showed a normal distribution. Categorical variables were compared using the chi-square test and Fisher's exact test, when necessary. Student's *t*-test and the Mann-Whitney test were applied to compare numerical variables. Logistic regression analysis was performed to identify variables that were independently associated with HCV-3. For all tests, $p<0.05$ was considered statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) 20.0 software (SPSS, Chicago, IL, USA).

Ethical considerations

This study was approved by the Ethics Committee of the Federal University of São Paulo.

RESULTS

A total of 306 patients with HCV-2 (n=50) and HCV-3 (n=256) were studied, and HCV-3 was more (84%) common. Subtype 2b (34%) was the most prevalent among patients infected with HCV-2 (n=50). Subtype 3a was identified in 244 (95%) of the 256 patients infected with HCV-3.

There was a predominance of men in the group of 306 (61%) patients, and the mean age was 47 ± 10 years. Risk factors for parenteral transmission were identified in 54% of the patients, with a transfusion risk identified in 37% of the patients and an intravenous drug use risk identified in 17% of the patients. The mean estimated duration of infection was 23±8 years. Arterial hypertension and diabetes mellitus were observed in 19% and 11% of cases, respectively. A BMI $\geq 25 kg/m^2$ was a characteristic of 56% of the patients; of these patients, 35% were overweight and 21% were obese. Elevated cholesterol and triglyceride levels were observed in 5% and 8% of cases, respectively. These data and the mean ALT, AST, GGT, and AP levels are shown in **Table 1**.

With respect to histology, an activity grade ≥2 was observed in 62% of the patients, and a fibrosis stage >2 was observed in 32% of the patients. Hepatic steatosis was present in 72% of the patients, whereas steatohepatitis was observed in only 3.5%.

A comparative analysis between HCV-2 and HCV-3 showed a younger age (p=0.002), less prevalence of arterial hypertension (p=0.03), higher serum albumin levels (p=0.01), more advanced stage of liver fibrosis (p=0.03), and higher frequency of steatosis in patients with HCV-3 (p=0.001). In addition, patients infected with HCV-3 tended to present a higher frequency of risk factors for parenteral transmission (p=0.06) and higher aminotransferase levels **(Table 2)**. A comparison of serum cholesterol and triglyceride levels between genotypes was not possible because these parameters were only altered in a small number of patients.

Age, arterial hypertension, serum albumin levels, liver fibrosis, and steatosis were the variables included in the multivariate logistic regression analysis. The results of the analysis indicated that all the variables, except the serum albumin levels, remained independently associated with HCV-3 **(Table 3)**.

TABLE 1 - Clinical, laboratory, and histological characteristics of patients with chronic hepatitis C caused by genotypes 2 and 3.

Characteristics	Total (n = 306)
Age (years)*	48 ± 10
Gender (%)	
male	61.0
female	39.0
Parenteral transmission risk n (%)	
blood transfusion	106 36.0
intravenous drug use	51 18.0
Duration of infection (years)*	23 ± 8.3
Diabetes *mellitus*	11.0
Arterial hypertension	19.0
BMI ≥25kg/m^2	56.0
AST (xULN)*	1.9 ± 1.5
ALT (xULN)*	2.5 ± 1.9
Alkaline phosphatase (xULN)*	0.6 ± 0.7
GGT (xULN)*	2.1 ± 2.1
Bilirubin (mg/dL)*	0.93 ± 0.84
Prothrombin activity (%)*	90.6 ± 13
Albumin (g/dL)*	4.2 ± 0.5
Platelets (n/mm^3)*	183,843 ± 66,575
Cholesterol ≥ 200/dL	5.0
Triglycerides ≥ 150mg/dL	8.0
Fibrosis (%)	
0-2	68.0
3-4	32.0
cirrhosis	21.0
Activity grade (%)	
0-1	38.0
2-3	62.0
Hepatic steatosis (%)	72.0
Genotype (%)	
2/2a/2b/2c/2a,c	3.0/4.0/6.0/1.0/2.0
3a	84.0

BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; ULN: upper limit of normal. *Values are expressed as the mean ± standard deviation. Histological analysis was performed according to the METAVIR system.

DISCUSSION

The distribution of HCV genotypes 2 and 3 and their subtypes varies according to geographic region. In the present study, which evaluated different aspects of HVC-2 and HVC-3, genotype 3 was more prevalent (84% of cases), whereas genotype 2 was identified in only 16% of cases. Genotypes 3a, 2a, and 2b were the most common subtypes.

Studies conducted in Brazil have shown a predominance of genotype 3 over genotype 2 among non-genotype-1 patients in all regions of the country. Campiotto et al.[29] studied the distribution of HCV genotypes and serotypes in different regions of Brazil and found genotype 3 in 30% of cases, whereas genotype 2 was identified in only 4.6% of the subjects.

An investigation of the demographic and epidemiological differences between patients infected with HVC-2 and HVC-3 showed that patients with HCV-2 had a higher mean age. In addition, parenteral transmission of the virus tended to be more frequent among subjects with HCV-3. From an epidemiological point of view, these findings appear to be related because genotype 3 is strongly associated with intravenous drug use, which is more frequent among young individuals. Katsoulidou et al.[30] studied 1,686 patients with chronic HCV in Greece and mainly identified genotype 3 among patients who contracted the infection through the use of intravenous drugs (58%). Taken together, these results suggest that genotype 3 was recently introduced in the population[3]. In fact, HCV genotype incidence data have shown a 1.5-fold reduction in the prevalence of genotype 2 between 1970 and 1990 and a 13-fold increase in that of genotype 3 over the same period[30].

The evaluation of clinical and laboratory differences between HVC-2 and HVC-3 showed that arterial hypertension was more frequent among patients with HCV-2. This finding might be due to the older age of these patients, as arterial hypertension is more frequent among older subjects. The laboratory parameters, with the exception of albumin, were similar for the two genotypes. Although the albumin levels were approximately normal in patients with HCV-3, they were slightly higher compared to those in patients with HCV-2. Despite this mathematical difference, this finding appears to be irrelevant from a clinical point of view.

Hepatic steatosis and advanced fibrosis were more frequent among patients infected with HCV-3 compared to patients with HCV-2. Studies have demonstrated a higher prevalence of steatosis in patients infected with genotype 3 compared to patients infected with other genotypes[31]. Rubbia-Brandt et al.[32] studied 101 patients with chronic hepatitis C, excluding patients with risk factors for nonalcoholic fatty liver disease, and they observed hepatic steatosis in 41% of the patients, irrespective of gender, age, or source of infection. Genotype 3 was more frequent than the other genotypes. The physiopathological mechanisms underlying the higher frequency of hepatic steatosis in patients with hepatitis C and genotype 3 suggest that the virus may have a cytopathic effect. According to Barba et al. [33], hepatic steatosis in hepatitis C appears to be the result of interference with the expression of genes involved in lipid metabolism.

TABLE 2 - Comparative analysis of clinical, laboratory, and histological variables in 306 patients with chronic hepatitis C genotypes 2 and 3.

Characteristics	Genotype 2 (n = 50)	Genotype 3 (n = 256)	p value
Age (years)[a]	52 ± 9	47 ± 10	0.002
Male*	56	62	0.4
Parenteral transmission risk*	42	56	0.06
Duration of infection (years)[a]	21 ± 9	24 ± 7	0.2
Diabetes*	8	11	0.5
Arterial hypertension*	30	17	0.03
BMI ≥ 25kg/m^2, yes/no*	17/18	83/82	0.7
AST (xULN)[a]	1.6 ± 1.4	2.0 ± 1.5	0.07
ALT (xULN)[a]	2.0 ± 1.8	2.6 ± 2.1	0.06
Alkaline phosphatase (xULN)[a]	0.8 ± 1.7	0.6 ± 0.3	0.1
GGT (xULN)[a]	2.2 ± 2.6	2.1 ± 1.8	0.6
Bilirubin (mg/dL)[a]	1 ± 1.7	0.9 ± 0.5	0.2
Prothrombin activity[a]	88 ± 17	91 ± 12	0.16
Albumin (g/dL)[a]	4 ± 0.4	4.2 ± 0.4	0.01
Platelets (n/mm^3)[a]	187,200 ± 60,427	180,977 ± 67,587	0.5
Fibrosis, 0-2/3-4*	81/19	65/35	0.03
Activity grade, 0-1/2-3*	64/36	53/47	0.1
Hepatic steatosis, yes/no*	49/51	75/25	0.001

BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; ULN: upper limit of normal. *Numbers are expressed as percentages. Data analysis was performed using the chi-square test, Student's *t*-test and Mann-Whitney test. [a] Mean ± SD. Histological analysis was performed according to the METAVIR system.

TABLE 3 - Final model illustrating multivariate logistic regression analysis of variables associated with HCV-3.

			95%CI	
Characteristics	p value	OR	lower	upper
Age (younger)	0.007	0.94	0.905	0.984
Arterial hypertension (less frequent)	0.03	0.37	0.145	0.943
Albumin (g/dL)	0.05	2.41	0.995	5.879
Fibrosis 3-4	0.03	3.02	1.058	8.624
Hepatic steatosis	0.005	3.38	1.457	7.845

HCV: hepatitis C virus; OR: odds ratio; 95%CI: 95% confidence interval.

Another finding of the present study was the association between HCV-3 and more advanced stages of fibrosis. Several risk factors have been associated with the histological progression of chronic hepatitis C, such as the duration of infection, age at infection, body weight, alcohol consumption, and double infection with HBV or HIV. However, the contribution of the HCV genotype to the progression of liver fibrosis is unclear. Bochud et al.[34] estimated the progression rate of fibrosis (METAVIR score/time of infection in years) in 1,189 patients and found a significant association between genotype 3 and advanced liver fibrosis. Genotype 3 was identified as an independent risk factor for fibrosis progression (OR=1.89, 95%CI: 1.37-2.61, p<0.001), as individuals with this genotype presented a higher progression rate compared to individuals with the other genotypes. In a systematic review of the literature conducted by Prosbt et al.[35], genotype 3 was associated with more rapid progression of liver fibrosis.

Possible explanations for the more advanced fibrosis in patients with genotype 3 include the presence of other factors associated with fibrosis progression, such as excessive alcohol consumption, greater pathogenicity of this genotype in terms of its ability to induce liver fibrosis, and the association with hepatic steatosis. Because alcohol abuse was excluded from the present study, this factor is not likely to explain the finding of more advanced fibrosis in this group of patients. It is therefore likely that characteristics of the genotype 3 itself, or the association of this genotype with steatosis are responsible for this finding. In this respect, studies have suggested that hepatic steatosis is associated with fibrosis progression in patients infected with genotype 3. Westin et al.[36] studied 98 patients with genotype 3 (n=25) and non-genotype 3 (n=64) HCV who were submitted to two liver biopsies and observed that the prevalence and degree of steatosis were strongly associated with genotype 3, irrespective of gender, age, BMI, and alcohol consumption. Faster fibrosis progression was more prevalent in patients with hepatic steatosis, an effect that was observed mainly in patients infected with genotype 3.

It can be concluded that differences exist between HVC-2 and HVC-3. Patients infected with HCV-3 were younger and had less frequent arterial hypertension; additionally, the prevalence of advanced liver fibrosis and hepatic steatosis was higher in these patients when compared to patients infected with HCV-2. These differences suggest that genotypes 2 and 3 should be analyzed separately, as they are genotypes with different clinical and histopathological characteristics.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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