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Chronic Hepatitis C Virus in the State of Piauí, Northeastern Brazil

Kelson Nobre Veras[1], Kelson Sousa Jacobina[2], Vítor Y. Rocha Soares[2], Marcela Amaral Avelino[2], Conceição de Maria S.R. Vasconcelos[3] and José Miguel L. Parente[4]

[1]Assistant Doctor, Health Municipal Foundation; [2]Medical Student of Federal University of Piauí; [3]Assistant Doctor, Hematology and Hemotherapy Center of the State of Piauí; [4]Supervising Doctor of Medical Residence Program in Gastroenterology, Federal University of Piauí; Teresina, Piauí, Brazil

Knowledge of genotype distribution of hepatitis C virus (HCV) has clinical importance due to genotype 1 lower response to treatment compared with genotypes 2 and 3. The goal of this survey was to describe clinical and laboratorial profiles of patients with chronic hepatitis C (CHC) in the State of Piauí, as well as to expand the overall awareness of the distribution of HCV genotyping in Northeast of Brazil. A retrospective cross-sectional study was carried out between April 1999 and August 2005. A total of 153 patients were included, 119 (77.8%) males and 34 (22.2%) females; mean age = 48.01 ± 9.11 years. We observed a homogeneous distribution between genotypes 1 (50.0%) and 3 (49.0%), while the most frequent subtype noticed was 3a (49.0%). The mean viral load among patients with subtype 1b (1,232,476 UI/mL) was significantly superior to the subtype 1a (391,204 UI/mL; p = 0.010) and to the subtype 3a (594,228 UI/mL; p = 0.047). The average levels of gamma-glutamiltransferase of genotype 1 (144 mg/dL) had statistical differences when compared to genotype 3 (74 mg/dL; p = 0.014). Most patients showed mild to moderate degrees of histopathological necroinflammatory activity and hepatic fibrosis (79.0% and 56.2%, respectively). We concluded that most candidates to treatment of CHC in the State of Piauí presented with clinically stable hepatic illness; the distribution of genotypes 1 and 3 was virtually homogeneous; and there was no significant demographic or clinical differences among genotypes or subtypes of HCV.

Key-Words: Epidemiology, hepatitis C virus, genotype distribution, chronic hepatitis C.

Hepatitis C Virus (HCV) is a single stranded RNA virus belonging to the *Flaviridae* family [1]. It is possible to classify HCV in genotypes (designated by Arabic numerals) and subtypes (designated by lower cases) using a method of amplification by reverse transcriptase-polymerase chain reaction (RT-PCR) of the NS5 region of HCV genome, followed by analysis with restriction fragment length polymorphism (RFLP) [2]. Nowadays, there are six main known genotypes designated by numbers 1 to 6; however, infection usually occurs by a single genotype. The identification of the genotypes has therapeutic and clinical implications due to a lower response to treatment shown by genotype 1 when compared with genotypes 2 and 3 [3-6].

World distribution of those several genotypes has some peculiarities. Genotypes 1, 2 and 3 are spread worldwide, although in different proportions according to the geographic area. Genotype 4 is restricted to Africa and Middle East, while genotypes 5 and 6 are uncommon, mainly encountered in South Africa and Hong Kong, respectively [7-10].

In the Northeast Region of Brazil, studies from the states of Bahia, Ceará and Rio Grande do Norte indicate that the main genotypes of HCV are types 1 and 3 [11-16]. Even though this genotype distribution resembles that found in Brazil as a whole, in fact these surveys had frequently presented only selective studies of specific groups such as drugs users, hemophiliacs, patients with chronic kidney disease and blood donors [15-21]. So, population surveys of HCV genotypic distribution are very needed in Brazil in order to know the real characteristics of this disease in this large country, as it was pointed out by Focaccia et al. [11]

The goal of this study was to describe clinical and demographic profile of patients with chronic hepatitis C in the state of Piaui as well as to enlarge the overall awareness of genotypic distribution of HCV in Northeast of Brazil.

Material and Methods

The Secretary of Health of Piauí offers free treatment to all patients with chronic hepatitis C who resides in Piauí. Since, data presented in this study can be considered as representative of persons with chronic hepatitis C in this area of our country.

This cross-sectional study was reviewed and approved by the Institutional Review Board of the Federal University of Piauí, Brazil. The study was carried out between April 1999 and August 2005.

Exclusion criteria were lack of laboratorial evidence of hepatitis C virus infection by immunological and/or polymerase chain reaction methods and hepatitis B virus or human immunodeficiency virus coinfection. Data from 153 patients were enrolled in the study. No patient had ever been treated before and only 12 patients had chronic kidney disease.

The following variables were analyzed: age, gender, genotype and subtype, viral load, serum aminotransferases (ALT and AST), total and direct bilirrubin, total protein, gamma-glutamiltransferase (γGT), creatinine, albumin, thyroid stimulant hormone, platelet count. Histopathological assays of liver specimens obtained by aspirative needle biopsies were analyzed for grading periportal or periseptal necroinflamatory

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Address for correspondence: Dr. Kelson Nobre Veras. Rua Taumaturgo de Azevedo 3443, apto. 1201. Zip code: 64001-340 Teresina, Piauí – Brazil. E-mail: kelsonveras@hotmail.com.

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activity and fibrosis according to Chronic Hepatitis Classification of Pathology and Hepatology Brazilian Societies [22]. Not all variables were available for all patients.

Statistical Analysis

The univariate Pearson's chi-square (χ^2) and Fisher's exact tests were used to measure differences in overall rates. Student's *t*-test and Levene's test were used to compare differences among continuous variables. The odds ratio (OR) and 95% confidence intervals (CI) were estimated. P values < 5% were statistically significant. Data were analyzed with the Statistics Packages of SPSS version 11.0 software (SPSS, Inc., Chicago, ILL) and MedCalc Version 9.3.2.0.

Results

A total of 153 patients were enrolled: 119 (77.8%) males and 34 (22.2%) females. Age varied between 16 and 74 years with mean ± SD age of 48.01 ± 9.11 years. Women were older than men with mean ± SD age of 52.76 ± 7.56 and 46.51 ± 9.06 years, respectively (p = 0.001).

Genotypes 1 (50.0%) and 3 (49.0%) had a similar distribution, while subtype 3a (49.0%) was the most frequent subtype observed. Amongst patients with genotype 1, thirty one (26.0%) presented with subtype 1a and 28 (24.0%) with subtype 1b (Table 1).

Table 1. Patients with Chronic Hepatitis C in the state of Piauí, Brazil, According to Genotyping.

Genotypes and Subtypes	Frequency N (%)
Genotypes 1	59 (50.0)
1a	31 (26.0)
1b	28 (24.0)
Genotypes 3	57 (49.0)
3a	57 (49.0)
Genotypes 2	1 (1.0)
2b	1 (1.0)
Total (n)	117 (100)

Laboratorial data are shown in Table 2. The mean level of viral load of patients with subtype 1b (1,232,476 IU/mL) was significantly higher to that of subtype 1a (391,204 IU/mL; p = 0.010) and to that of subtype 3a (594,228 IU/mL; p = 0.047). The mean levels of γGT showed statistical differences among patients with genotype 1 when they were compared with patients with genotype 3 (144 mg/dL and 74 mg/dL, respectively; p = 0.014). There were no statistically significant differences within other laboratorial exams between patients with subtypes 1a, 1b and 3a (Table 02).

Histopathology of hepatic biopsies showed that most patients (79.0%) presented mild to moderate degrees of necroinflamatory activity, while 56.7% presented with mild to moderate degrees of fibrosis (Table 3). The only significant difference found between genotypes and degree of fibrosis was a greater risk to septal fibrosis – F3 – in genotype 3 (Table 4).

Discussion

The present study indicates that HCV genotypes 1 and 3 were the most prevalent with an identical distribution, while genotype 2 was found only in one patient. In the state of Ceará, a neighboring state of Northeastern Brazil, a previous study also found a similar distribution, i.e., 50.0% for genotypes 1 and 3 among 22 patients under hemodialysis [15]. However, our results differ from the study carried out by Focaccia et al., involving a greater number of patients from several regions of Brazil, which found 64.0% infected with genotype 1, and 33.0% for genotype 3 [11]. Similarly, Vasconcelos et al., also in the state of Piauí, found 74.1% and 61.0% for genotype 1 and 22.2% and 36.0% for genotype 3, among patients under hemodialysis and blood donors, respectively [23].

Other issues indicate a predominance of genotype 1 in Southeastern Region of Brazil [11,15,17,18,24,25]. Its prevalence is around 70.0% in the states of Rio de Janeiro and São Paulo, and 84.1% in Minas Gerais [18]. In Northeastern Region, many surveys were carried out in the state of Bahia involving a great number of cases. The genotypic variation follows the pattern of the rest of Brazil, with genotype 1 corresponding about 70.0% of the individuals infected [11-13]. On the other hand, some studies from South Region indicate a profile of genotypic distribution closer to that found in our study. There, genotype 3 has already been described as the predominant genotype in the state of Rio Grande do Sul [25]. Taken together, data from Paraná and Rio Grande do Sul indicate a major role for genotype 3 in South Region (44% of cases) as compared with other regions of Brazil, though genotype 1 still predominates (51% of patients) [11].

Genotype 3 has been associated with transmission through illicit injection-drug use. In Rio de Janeiro, genotype 3 was more frequently found between intravenous drugs users as compared with blood donors and hemophilic patients [15]. In France, besides an independent association between genotype 3 and injection-drug use, its frequency increased between 1970 to 1990, when the ratio of infected patients rose from 35.1% to 54.8% [26]. The present study is not intended to analyze the association between genotypes and ways of transmission of hepatitis C virus. However, there are many anecdotal accounts of medical use of intravenous stimulants in this population, whose users shared syringes or used inadequately sterilized glass syringes. Thus, it can be deduced that this habit could have contributed to a large dissemination of genotype 3 in our region as other authors have already suggested [17,27].

The mean age of our patients was about 48 years, similar to what has been described in other studies in Brazil [11,13,17,24,28]. There was no difference between mean ages in patients with genotypes 1 and 3 (48.0 and 47.7 years old, respectively), similar to what was found by Bassit et al. in São Paulo [24]. In a survey from Bahia, the average age of the patients infected with genotype 3 was inferior to age of patients with genotype 1 (38 and 55 years, respectively) [12]. In our study, ages of females patients were higher than those of

Table 2. Patients with chronic hepatitis C in the state of Piauí, Brazil, according to biochemistry, genotypes and subtypes aspects.

Variables	1	1a Mean ± SD (n)	1b Mean ± SD (n)	3a Mean ± SD (n)	Mean ± SD	P value
Viral Load (UI/mm^3)	781,062 ± 974,778,3 (41)	391,204,4 ± 412,658,2 (22)	1,232,476 ± 1,229,638,9 (19)	594,227,6 ± 720,985,9 (40)	647,480,07 ± 815.839,04	0.010† 0.047¶
AST (U/l)	88.7 ± 51.2 (58)	91.1 ± 57.9 (31)	85.9 ± 43 (27)	93.6 ± 52.3 (56)	94.51 ± 57.27	> 0.050*
ALT (U/l)	121.3 ± 73 (59)	122.3 ± 79.1 (31)	120.3 ± 67,2 (28)	121.12 ± 62.5 (56)	122.55 ± 71.70	> 0.050*
Total bilirrubin (mg%)	1.3 ± 0.9 (40)	1.3 ± 0.9 (23)	1.3 ± 1 (17)	1.1 ± 0.5 (40)	1.20 ± 0.83	> 0.050*
Direct bilirrubin (mg%)	0.6± 0.5 (40)	0.5 ± 0.5 (23)	0.6 ± 0.6 (17)	0.4 ± 0.2 (40)	0.54 ± 0.57	> 0.050*
Total protein (g%)	7.9 ± 0.8 (38)	7.3 ± 0.7 (21)	6.9 ± 0.9 (17)	7.0 ± 1 (39)	7.05 ± 0.95	> 0.050*
Albumin (g%)	4.1 ± 0.6 (41)	4.1 ± 0.6 (24)	4.2 ± 0.7 (17)	4.2 ± 0.6 (45)	4.15 ± 0.67	> 0.050*
Gama-GT (U/L)	143.8 ± 140 (34)	141.6 ± 151.6 (19)	146.5 ± 129.1 (15)	74.5 ± 75.2 (33)	108.88 ± 109.49	0.014§ 0.037$^\#$
Platelets (U/mm^3)	174,176.5 ± 46,195.1 (51)	167,620.7 ± 47,465.4 (29)	182,818.2 ± 44,038.6 (22)	178,485.7 ± 66,307.4 (49)	178,158.91 ± 62,221.31	0.050*
TSH (U/mm^3)	1.6 ± 1.1 (35)	1.7 ± 1.2 (21)	1.3 ± 0.8 (14)	1.6 ± 0.8 (40)	1.59 ± 0.91	> 0,050*

*Comparison between subtypes and between genotypes; †Comparison between subtypes 1a and 1b; ¶ Comparison between subtypes 1b and 3a; §Comparison between genotypes 1 and 3; #Comparison between subtypes 1a and 3a; Gama-GT = gamaglutamitransferase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; TSH = thyroid-stimulating hormone.

Table 3. Patients with chronic hepatitis C in the state of Piauí, Brazil, according to histological features.

Histological Features	Frequency	Percentage (%)
Fibrosis		
F0	21	18.6
F1	23	20.4
F2	20	17.7
F3	28	24.8
F4	21	18.6
Total (n)	113	100.0
Periportal Necroinflamatory Activity		
Mild (0 to 2)	21	20.0
Moderate (3)	62	59.0
Severe (4)	22	21.0
Total (n)	105	100.0

males (53 and 46 years, respectively; p = 0.001). These data are in accordance with results found in another Brazilian study.[11] Looking at gender, we found a predominance of males (78.0%), and this result is similar to that observed in other regions of Brazil [11,13,17,23,24]. There was no association between genotype and gender, and this finding is in agreement with other studies in national literature [13,24].

The mean levels of bilirrubins, total proteins, albumin and platelets were within normal ranges, showing a profile of patients with compensated hepatic illness. Mean levels of serum aminotransferases and gamaglutamiltransferase of our patients revealed only mild changes as it is common in patients with CHC [29,30]. According to genotyping, only patients infected with genotype 1 showed high mean levels of gamaglutamiltransferase (p = 0.019). Gamaglutamiltransferase is known as a sensible but nonspecific marker of hepatobiliar

Table 4. Association between viral genotypes and hepatic histopathological findings.

Hepatic histopathology	Gentotype 1	Genotype 3	Total	Odds ratio (95% confidence interval)	P value
Fibrosis					
F0	11 (64.7)	6 (35.3)	17	2.17 (0.73 – 6.42)	> 0.05
F1	11 (50)	11 (50)	22	1.05 (0.41 – 2.71)	> 0.05
F2	12 (66.7)	6 (33.3)	18	2.43 (0.83 – 7.10)	> 0.05
F3	7 (28)	18 (72)	25	0.31 (0.11 – 0.82)	0.021
F4	8 (44.4)	10 (55.6)	18	0.80 (0.29 – 2.23)	> 0.05
Total	49	51	100		
Periportal necroinflamatory activity					
Mild (0 – 2)	9 (50)	9 (50)	18	1.01 (0.39 – 3.03)	> 0.05
Moderate (3)	24 (44.4)	30 (55.6)	54	0.69 (0.30 – 1.57)	0.41
Severe (4)	12 (57.1)	9 (42.9)	21	1.58 (0.60 – 4.20)	0.46
Total	45	48	93		

illness [31], and it is associated to advanced hepatic fibrosis [17]. However, there was no association between genotype 1 and degree of fibrosis in this study. Therefore, we could not explain the raised levels of gamaglutamiltransferase found in genotype 1 in our survey. Other possible confusing variables that had not been evaluated in this study, particularly intake of alcohol [32], could explain the results presented by us. Different from Codes et al. [13], we did not find thyroid dysfunction among patients with HCV genotypes 1 and 3.

The majority of our patients had mild to moderate degrees of periportal or perisseptal necroinflammatory activities, i.e., between 0 and 3 according to classification of Brazilian Societies of Pathology and Hepatology, while half of them presented with mild to moderate degrees of fibrosis. Similar histopathological profiles of patients with chronic hepatitis C was shown in other regions of Brazil [13,17,23,25]. In spite of this, some patients had cogitated their treatment only in advanced phase of fibrosis, and hepatic cirrhosis was already present in almost 20.0% of them before beginning of treatment.

No national study had found an association between degree of fibrosis and genotypes of HCV. In fact, this topic seems to be settled as well as that genotypes do not predict severity of hepatitis C [7,33]. Nevertheless, our findings showed a significant lower risk to a high degree hepatic fibrosis (F3) with genotype 1 as compared with genotype 3 (OR = 0.31; CI: 0.11 to 0.82; p = 0.02). However this is a subgroup analysis of a small number of patients.

A caveat in our survey is that genotype frequencies may vary when comparing population and referral center genotypic distribution data as ours. Zarife et al. [34] found in Salvador that genotype 3 was the most common genotype (53.3%), followed by genotypes 1 (40%) and 2 (6.7%). However, when comparing these observations with data from hospital and ambulatory patients from the same city, genotype 1 was the most frequent, followed by genotypes 3 and 2.

In conclusion, our analysis of candidates to treatment of CHC in the state of Piauí, Northeast of Brazil, showed preponderantly male patients, in the course of the fifth decade of life, presenting clinically compensated illness; the majority had mild to moderate degrees of histological activity and fibrosis; genotypes 1 and 3 were practically homogeneous, and genotype 2 was rare; significant demographic and clinical differences had not been shown between genotypes.

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