Liver Histological Alterations in Patients With Chronic Hepatitis C and Normal ALT Levels in the City of Salvador, Northeast-Brazil

Nelma Pereira de Santana[1], Luiz A.R. de Freitas[2], André Castro Lyra[1], Raymundo Paraná[3], Genoile Santana[1], Christian Trepo[4] and Luiz G.C. Lyra[1]

Gastro-Hepatology Service Hospital Prof. Edgard Santos from Federal University of Bahia, Gastro-hepatology Service of Hospital São Rafael[1]; CPqGM Oswaldo Cruz Foundation, Fiocruz, Pathology Department, Federal University of Bahia[2]; Post-graduate course in Medicine and Health Federal University of Bahia[3]; Salvador, BA, Brazil; Viral Hepatitis Laboratory, Unit 271 INSERM[4], Lyon, France

Patients with chronic hepatitis C can have variable clinical progression. Hepatic histological alterations appear to be milder in asymptomatic subjects who have persistently normal ALT levels. Aims: To evaluate the severity of histological liver alterations in blood donors with normal and elevated ALT levels. Methods: We evaluated volunteer blood donors from the main blood bank of the city of Salvador-Brazil. Those who were anti-HCV positive were invited to participate in the study. Serum ALT and AST levels were measured at two time points, two months apart. Donors were divided into two groups: group I, individuals with ALT ≥ 1.5 times the upper limit of normal in at least one time point and group II, individuals with normal or near normal ALT, at both time points. Results: We evaluated 30,232 blood donors and 528 (1.7%) of them were anti-HCV positive. Eighty-two attended our service and HCV infection was confirmed in 66 individuals. Male gender predominated in both groups; the mean age was 36 for group I, and 33 for group II. Tattoos and intravenous illicit drug use were frequently-encountered risk factors. Liver biopsy was done in 43 subjects. Among donors with elevated ALT, two (10%) had minimum alterations, while in group II normal liver or minimum alterations were observed in six (26%) subjects. Chronic hepatitis or cirrhosis was encountered in 35 (81%) individuals: three (15%) and five (21%) subjects had chronic hepatitis without inflammatory activity, 10 (50%) and 11 (48%) had minimum to moderate activity and five (25%) and one (4.3%) had cirrhosis, in groups I and II, respectively (P was not significant). Conclusions: The prevalence of anti-HCV among this population of volunteer blood donors was 1.7%, and these subjects had few liver histological alterations or chronic hepatitis and cirrhosis. Liver injury severity was significant in patients with elevated ALT, however subjects with normal levels may also present chronic hepatitis and cirrhosis.

Key Words: ALT levels, liver alterations, HCV, Northeast-Brazil.

Abbreviations: HCV (hepatitis C virus), ULN (upper limit of normal), HUPES (University Hospital Prof. Edgard Santos).

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Address for correspondence: Dr. Luiz G. Costa Lyra. Av. Juracy Magalhães Jr. 1855/501, Rio Vermelho, Salvador, Bahia, Brazil. Zip code:41940-060. Tel 55-71-2760-378/ 55-71-350-4664 /Fax 55-71-276-2106 or 55-71-399-6266. Email: lglyra@atarde.com.br or gastro.hepatologia@hsr.com.br.

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Hepatitis C virus (HCV) infection has a universal distribution. The diagnosis of HCV infection is made by detection of anti-HCV antibody and HCV-RNA in serum. Confirmatory tests, such as the RIBA assay, can be used to confirm the specificity of anti-HCV antibody and to evaluate false positive results. HCV infection has peculiar clinical and evolutive characteristics, most of the time with a long asymptomatic period and normal aminotransferase

levels. Subjects infected by HCV may progress with either normal or elevated ALT levels. Cohort studies have shown that the natural history of this disease is characterized by slow progression, with development of cirrhosis occurring after 20 to 30 years of infection, especially in children and young women [1-3]. In men, or in those with associated risk factors, such as alcoholism, immunosupression, or co-infection with hepatitis B virus or HIV, the development of cirrhosis is faster.

Around 30% of patients with chronic HCV infection have elevated ALT levels, 40% have ALT levels close to the upper limit of normal (<2 times the upper limit of normal) and 30% have normal ALT [4]. Several authors have evaluated hepatic histological alterations in asymptomatic carriers according to ALT levels and have found more severe hepatic injury in those with elevated ALT; however cirrhosis was detected in a small number of individuals with normal ALT [5-7]. Other investigators could not find any differences in terms of HCV histological alterations according to the level of ALT [8]. The faster or slower potential of HCV infection progression and ALT levels have implications for the management of the disease, including use of liver biopsy and therapy with the currently-available drugs. The severity of hepatic alterations in asymptomatic individuals infected with HCV has been studied in different regions of the world. This is the first study in Salvador, Bahia, Brazil that has evaluated the level of ALT and the liver histological findings in patients with chronic HCV infection.

Material and Methods

We evaluated volunteer blood donors from the main blood bank of the city of Salavador, Bahia, Hemocentro da Bahia (HEMOBA), and from the Hospital São Rafael between March 92 and June 94. Those who were positive for anti-HCV antibody by ELISA II were invited by letter to attend the Hepatology Outpatient Service of the University Hospital Prof. Edgard Santos (HUPES). We only included subjects with no symptoms of acute or chronic liver disease who were positive for anti-HCV in serum and negative for other serological tests, such as HbsAg, anti-HIV and anti-HTLV-I. They were interviewed and answered a questionnaire about risk factors for HCV infection: tattoos, parenteral use of illicit drugs, and previous blood transfusion. Subjects with a history of alcohol abuse defined as ingestion of alcoholic beverages during four or more days in a week were excluded. Anamnesis and physical examination were performed in all individuals.

Biochemical analysis was carried out in the central laboratory of the University Hospital; serum ALT and AST levels were measured at two consecutive time points two months apart. Total proteins and albumin, prothrombin time, and alkaline phosphatase were also evaluated. Anti-HCV positivity was confirmed by the RIBA-III test (Chiron, Emeryville, CA), which uses C100-3, C33c, C22-3 and NS5 as antigens. HCVRNA was detected by RT-PCR in the Hepatitis Laboratory, Unit 271 of INSERM, Lyon, France.

Donors were divided into two groups: Group I – Individuals with ALT ≥ 1.5 times the upper limit of normal in at least one time point. Group II – Individuals with ALT < 1.5 times the upper limit of normal at both time points.

All subjects who were diagnosed with HCV infection were asked to undergo liver biopsy. Hepatic tissue fragments were fixed in 10% formaldehyde and subjected to the following staining: a) hematoxiline and eosine; b) red picrosirius; c) Gomori's silver impregnation stain; d) PAS with or without diastasis; and e) Perls (Prussian blue reaction). The same pathologist evaluated all of the hepatic tissue fragments, without his knowledge about serologic and biochemical results.

The histological analysis took into account the Knodel classification and the criteria proposed by Gudat and Bianchi for the evaluation of chronic hepatitis [9,10]. A semi-quantitative graduation system was established, subdivided into minimum, moderate and severe activity, indicating the degree of severity of the necro-inflammatory injury and fibrosis. Numerical indexes were adopted, taking into account the severity of portal inflammation, piece-meal necrosis, necro-

inflammatory activity of parenchyma, and fibrosis. Features analyzed included macro or micro vacuolar steatosis, presence of lymphocyte aggregates or lymphoid folliculate in portal spaces, presence of ductal lesions, alterations possibly related to alcoholism and the use of intravenous drugs.

Mild portal chronic inflammation, discrete sinusoidal infiltration with minimum lobule inflammation, activation of Kupfer cells and diffuse thickening of reticulinic trama, isolated hepatocyte apoptosis (Councilman) and hepatocytary centrolobular balloonization were considered "minimum histological alterations".

Statistical analysis was done using the Chi-square test or Fisher's exact test to evaluate differences between proportions and the Student's t test for comparison of means. Data were analyzed with EPI-INFO program version 5.0 1b; P values < 0.05 were considered statistically significant.

Results

We evaluated 30,232 blood donors from two blood banks. Five hundred twenty-eighty (1.7%) were anti-HCV positive. Among the 82 subjects who attended the HUPES Hepatology Outpatient Service, all were subjected to an HCV RIBA-III test. Sixty-six of them (80.5%) were confirmed to be HCV positive by RIBA-III and were included in analysis. Among the remaining 16 patients who were not RIBA III positive, 13 (15.9%) were negative and in 3 cases (3.7%) the test was indeterminate (Table1).

All 66 donors included in the final analysis were asymptomatic; 59 (89%) were male and 7 (11%), female. Group I (elevated aminotransferases) comprised 25 (38%) individuals and Group II, 41 (62%). Mean age in group I was 36; hepatomegaly was detected in four (16%) individuals and peripheral signs of liver disease in two (8%). Mean age in group II was 33, hepatomegaly was detected in one subject (2.4%) and peripheral signs of liver disease in one (2.4%) (Table 2).

Risk factors for HCV infection were identified in 41 (62%) out of the 66 individuals studied; 20 of them (49%) had tattoos, two (4.9%) had a previous history of blood transfusion and 19 out of 41 (46%) had a history of intravenous illicit drug abuse. There was no significant difference between groups I and II in terms of HCV risk factors (Table 3).

Protein fraction analysis of anti-HCV antibodies showed reactivity of 95.5%, 98.5%, 97% and 74.% for anti-C100-3, anti-C33-c, anti-C22-3 and anti-NS5, respectively. There was a trend for individuals of group I (elevated ALT) to have a greater frequency of anti-NS5, compared to donors from group II (normal ALT) (88% vs. 66%) (Table 4).

Forty-three (38 men and 5 women) individuals underwent liver biopsy, 20 from group I and 23 from group II. HCV RNA was positive in 23 patients of Group II and in nine of group I. HCV RNA could not be carried out in 11 patients of group I; however, all were RIBA-III positive.

Histological analysis of the 43 subjects who underwent liver biopsy showed a normal liver in one (2,3%) and seven (16%) had minimum histological alterations. Liver injury related to chronic hepatitis and/or cirrhosis were described in 35 (81%) donors; 8 (19%) of them had chronic hepatitis with no evidence of inflammatory activity (without interface hepatitis) and 21 (49%) had chronic hepatitis with minimum (16 donors) to moderate (five donors) activity. No severe inflammatory activity was observed in any case. Cirrhosis was detected in six (14%) individuals (Table 5).

There was no blood donor in Group-I with a normal liver; two (10%) had minimum histological alterations; three (15%) had chronic hepatitis without activity (interface hepatitis); 10 (50%) had chronic hepatitis with histological activity and 5 (25%) had established cirrhosis.

In group II, one subject (4.a 3%) had normal liver; 5 (22%) had minimum histological alterations, five (22%) had chronic hepatitis without inflammatory activity; 11 (48%) individuals had chronic hepatitis and activity, and 1 (4.3%) had cirrhosis. There was no significant difference in the liver alterations between the two groups (Table 5).

Table 1. Frequency of anti-HCV in serum in blood donation candidates

Characteristics	N (%)
Number of donors	30,232
Number of anti-HCV ELISA II positive	528 (1.7%)
Number of donors who attended HUPES	82
Number of donors who were subjected to RIBA-III	82
Number of donors who were RIBA III positive	66 (80.5%)
Number of donors who were RIBA III negative	13 (15.9%)
Number of donors who were RIBA III undetermined	3 (3.7%)

Table 2. Characteristics of the 66 anti-HCV and RIBA III positive individuals

Characteristics	Group I (N=25)	Group II (N=41)	Total (N=66)
Gender M/F	23/2	36/5	59/7
Mean age (years)	36	33	34.1
Hepatomegaly	4	1	5
Peripheral signs of liver disease	2	1	3
Number of individuals who underwent liver biopsy	20	23	43

Group I: ALT ≥ 1.5 times the upper limit for normal. Grupo II:ALT < 1.5 times the upper limit for normal.

Table 3. Distribution of risk factors for HCV infection in each group

Risk factors	Group I (ALT≥1.5 ULN.) N = 25	Group II (ALT<1.5 ULN.) N = 41	Total N (%) N = 66	P
Tattoo	5 (20.0%)	15 (36.6%)	20 (30.0%)	0.251
Blood transfusion	2 (8.0%)	0	2 (3.0%)	0.139
Use of IV drugs	9 (36.0%)	10 (24.4%)	19 (28.8%)	0.465

ULN = upper limit of normal.

Histological findings showed lymphoid follicles in 21 (48%) donors, 11 (52%) of them from group I and 10 (48%) from group II; ductal lesions were present in 19 (44%) individuals, 10 (53%) of group I and 9 (47%) of group II; macro or micro vacuolar steatosis were found in 28 (65%) individuals, 14 (50%) of group I and 14 (50%) of group II.

The use of intravenous illicit drugs was reported by 19 donors; seven of them had macrophages in portal spaces phagocytosing a dark brown and granulous pigment, which was refringent to polarized light. Only two donors had alterations consistent with alcoholic disease of the liver, characterized by the presence of Mallory corpuscles and micro nodular cirrhosis.

Table 4. Distribution of antibodies for anti-HCV according to the serum level of ALT

Antibody antiVHC	Group I ALT≥1.5 (x ULN) N (%)	Group II ALT< (x ULN) N (%)	P
Anti-C100-3	25 (100.0%)	38 (92.7%)	0.283
Anti-C33c	25 (100.0%)	40 (97.6%)	1.000
Anti-C22-3	25 (100.0%)	39 (95.1%)	0.522
Anti-NS5	22 (88.8%)	27 (65.9%)	0.088
Total	25	41	

ULN = upper limit of normal.

Table 5. Histological hepatic alterations found in both ALT-level groups

Histological findings	N (%)	Group I ALT≥ 1.5 (x ULN) N = 20 (%)	Group II ALT< 1.5 (x ULN) N = 23 (%)	P
Normal liver	1 (2.3%)	0	1 (4.3%)	1.000
Minimum histological alterations	7 (16.3%)	2 (10.0%)	5 (21.7%)	0.420
Chronic hepatitis without activity, without hepatitis in the interface	8 (18.6%)	3 (15.0%)	5 (21.7%)	0.704
Chronic hepatitis with activity, with hepatitis in the interface	21 (48.8%)	10 (50.0%)	11 (47.8%)	0.870
Cirrhosis	6 (14.0%)	5 (25.0%)	1 (4.3%)	0.814
Total	43	20	23	

ULN = upper limit of normal.

Discussion

It is estimated that approximately 150 million people are infected by HCV worldwide. Cohort studies that evaluated the natural history of the disease have shown that HCV infection generally has a slow progression, with a long asymptomatic period, and only after 20 years of evolution does cirrhosis occur. Cases of individuals, who became infected and remained viremic without developing cirrhosis for as long as 50 years, have also been reported. This is observed more frequently in young women and children. On the other hand, in elderly male patients who have associated co-morbid factors, such as HIV and HBV co-infection or alcoholism, the development of cirrhosis is premature, generally occurring less than 10 years after HCV infection. It is estimated that hepatocellular carcinoma occurs at a frequency of 0% to 3% per year, once cirrhosis is established. Therefore, preventing cirrhosis through the interruption of viral replication, or eliminating the virus, becomes mandatory. Although there are established criteria for the current treatment of chronic HCV infection, there are also controversies among specialists. The treatment of patients with minimum hepatic alterations or with normal ALT is not yet widely recommended; it is still being debated. On the other hand, it has been observed that most patients infected by HCV have normal ALT at initial presentation, which explains the numerous cases of anti-HCV-positive blood-donor candidates found in our study.

The groups with normal and elevated ALT levels in this study were similar in terms of variables that could interfere with the evolution of HCV infection and severity of liver injury. In both groups there was a predominance of male gender, and mean age was similar. In patients with normal ALT, most studies have shown a preponderance of women, although other investigators have not confirmed differences related to gender [8,11]. In our study this evaluation may be impaired since most of blood donors were males. A history of intravenous illicit drug use and tattoos were risk factors reported by the study population. This suggests that information about illicit drug use is omitted in the selection process for blood donation. It is possible that Brazilian public hospitals policy, such as an obligation to donate blood in order to have the right to visit relatives admitted to the hospital, is one of the motives for inadequate information in the selection process for blood donation.

The frequency of anti-HCV in blood donors varies from about 1% to 2%, according to the geographic site and the type of population studied, except in places where there is a large prevalence of HCV, like Egypt. We found a prevalence of 1.7% anti-HCV in 30,232 blood donors evaluated in HEMOBA and in the Hospital São Rafael in Salvador, BA, which is similar to what has been described in other regions of Brazil. The specificity of both test second and third generation ELISA is greater than 95% in immunocompetent individuals; thus the RIBA test is not necessary for routine clinical practice and should rather be used as a research tool. Sixty-six (80.5%) of the 82 anti-HCV positive blood donors who attended our outpatient service were RIBA-III positive as well. Among protein fractions, NS5, which is related in part to viral replication, predominated in the group of patients with elevated ALT, although this predominance was not statistically significant.

Approximately 30% of patients with chronic hepatitis had normal ALT levels and 40% had ALT $\leq$ 1.5 to 2 times the upper limit of normal [4]. Even though most of these patients had only mild inflammation or fibrosis, progression to cirrhosis has been reported in several studies [8,11,12].

In our study, among 43 subjects who underwent liver biopsy, HCV RNA could not be performed in 11 patients, all from group I (elevated ALT). All of these were RIBA-III positive, suggesting they were infected by HCV. Histological analysis showed a trend towards milder alterations in blood donors who had normal ALT levels (group II). Normal liver or minimum histological alterations was described in 6 (26%) of the 23 subjects with normal ALT levels, while none of the 20 blood donors with elevated ALT had a normal liver and only two (10%) had minimal alterations. On the other hand, more severe hepatic injury, such as chronic hepatitis with abnormal activity and/or cirrhosis were predominant in the group with elevated ALT, corresponding to 75% of the biopsies, five (25%) of them with established cirrhosis. Other investigators have compared subjects with normal ALT levels to patients with elevated ALT [5-7]. Overall, they found milder hepatic injury in patients with normal or near normal ALT levels. In one of these studies, the authors encountered a normal liver in 15% of the subjects; however cirrhosis was not reported. In another, all subjects had minimum histological alterations and none had a normal liver or cirrhosis. Montalto et al. described the presence of chronic hepatitis and/or cirrhosis in 44% of patients [12]. Other authors have found cirrhosis, in 26% and 44% of cases, respectively, but have not detected differences between individuals with normal ALT versus elevated ALT [8,11].

We found milder alterations, such as minimal histological findings, in individuals with normal or nearly normal ALT levels; nevertheless, 11 patients (48%) had chronic hepatitis with minimum to moderate activity and 1 (4.3%) had cirrhosis (Figures 1 and 2).

The concept of normal ALT is controversial, considering the peculiar aspect of the evolution of HCV infection. Patients may have normal ALT levels for several months and afterwards present with elevated ALT. It has yet to be defined how long the period of follow-up should be to characterize that a patient has normal ALT. Some authors suggest monthly determinations of ALT for a period of 6 to 12 months. In our study, we utilized two ALT measurements two months apart. We are aware that if we had used more

Figure 1. Dense mononuclear inflammatory infiltrate of the portal-space found in a patient with normal ALT. The limit between parenchyma and mesenchyma is not clear; the infiltrate invades the parenchymal rim (hepatitis interface) surrounding the hepatocytes. There is micro and macro vacuolar steatosis, besides a discreet intra-sinusidal lymphocytary infiltrate (HE x 100).

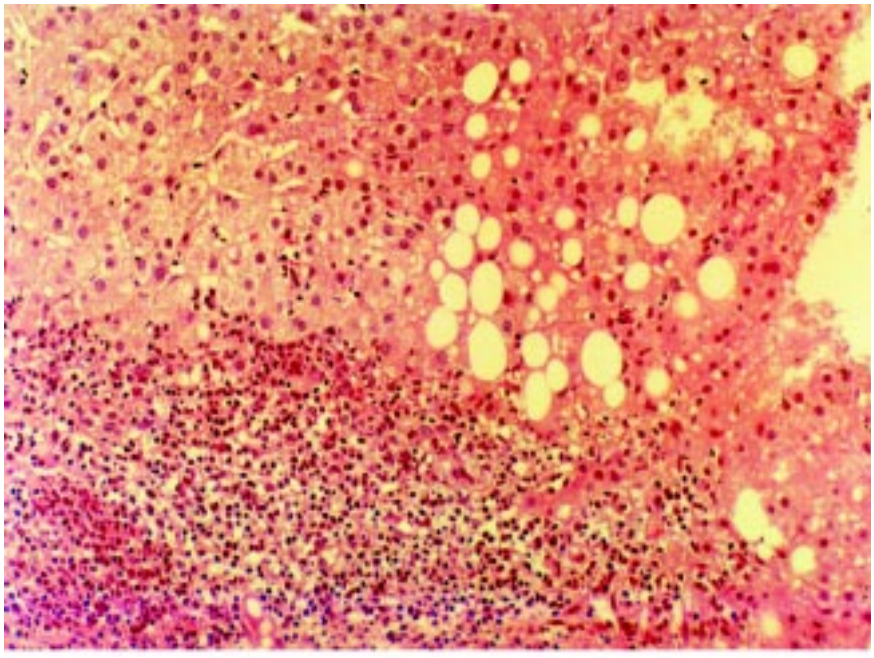
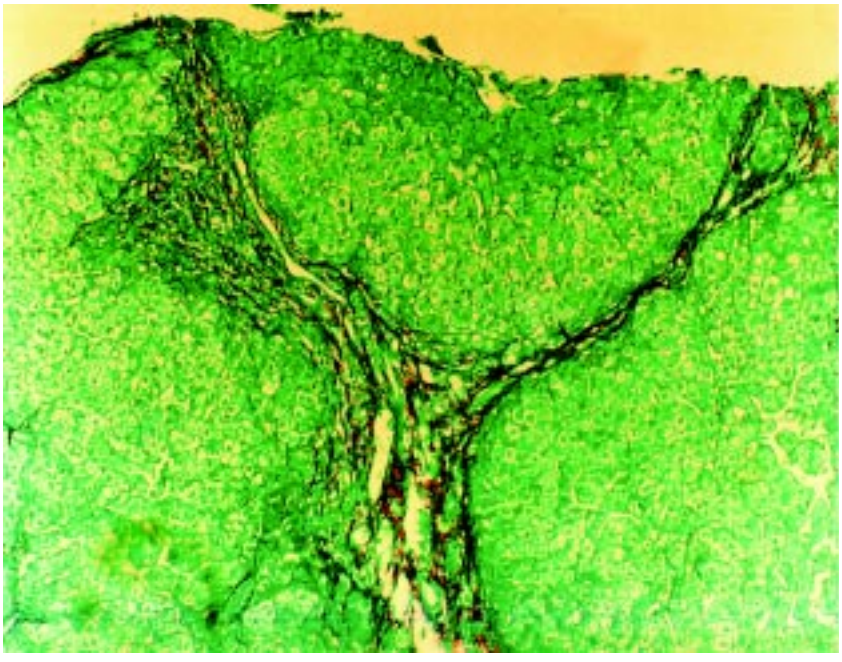


Figure 2. Presence of parenchymatous regenerative nodules delimitated by thin fibrotic septa (cirrhosis) in a patient with normal ALT levels. (Picrosirius x 100).



ALT measurements we would have had a greater chance to detect elevated ALT, however the characteristics of the blood donor candidates suggest that they were individuals with persistently normal ALT levels, since they asymptomatic and most of them had a normal physical examination.

In conclusion, the prevalence of anti-HCV antibodies among 30,232 volunteer blood donors from the city of Salvador, Brazil was 1.7%. Tattoos and the use of intravenous illicit drugs were risk factors for HCV infection, suggesting omission of this information during the selection process of blood donation. Histological alterations in the liver ranged from minimum alterations to chronic hepatitis and cirrhosis. Liver injury appeared to be more severe in the group of patients with elevated ALT levels, in which there were no subjects with normal livers. In blood donor candidates with normal or near normal ALT, we found mild hepatic histological alterations more frequently in the latter than in the former group; nevertheless chronic hepatitis and even cirrhosis were encountered in a few subjects. This study adds to knowledge about liver histological alterations found in individuals with hepatitis C and normal ALT. Further investigations in this subgroup of patients are necessary in order to determine if these subjects should be treated with the currently available drugs.

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