

PATIENTS WITH CHRONIC HEPATITIS C AND NORMAL TRANSAMINASES

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SUMMARY

Hepatitis C virus infection evolves progressively persisting in the majority of patients (85%). Most patients have high ALT (alanine aminotransferase) levels and approximately 25% normal ALT. The latter are usually female and there is no association between genotype and severity of hepatic lesion. Histologic analysis usually shows small lesion and absence or low amount of fibrosis, despite cirrhosis having been reported. Aiming at assessing prevalence, demographic, genotypical and anatomopathological characteristics in patients with normal ALT levels, we have carried out a study of 68 chronic hepatitis C patients between January 1997 and April 2000. There was a prevalence of 13.8% chronic hepatitis C patients with normal ALT levels, 45.6% of which were male and 54.4% female, the mean age being 38 +/- 13 years. We found a predominance of genotype 1 in 84.7% of the patients, genotype 2 in 6.8% and genotype 3 in 10.7%. In 52.9% of the cases liver biopsies revealed liver reaction, periportal activity score 0-1 was observed in 85.3% of the patients and score 2-4 was seen in 14.7%. Structural activity score 0-1 was seen in 73.5% of the patients and score 2-4 in 26.5% of them. Periportal activity ≥ 2 and structural activity > 1 was seen in 29%, but steatosis was not seen in 73.5%. Our results suggest the need to revisit for liver biopsy practice in patients with Chronic Hepatitis C and normal transaminases.

KEYWORDS: Hepatitis C; Normal transaminases; Biopsy.

INTRODUCTION

In spite of new hepatitis C virus (HCV) infections having declined in the last decade in most developed countries, chronic hepatitis C is still a major public health concern.

An estimated 200 million people are currently chronically infected with HCV²⁵. 75% of these patients have elevated ALT levels and in 20 to 30% ALT levels are normal²⁹. HCV has become one of the major causes of elevated aminotransferases in asymptomatic patients.

Serum aminotransferases testing has been widely used as a marker of liver disease activity, however, the literature has objected to this finding, because liver biopsies carried out in patients with normal transaminases show some degree of hepatic lesion, although, in most cases, histopathology of the lesion is mild and progression to fibrosis lower than in patients with elevated ALT, thus, denying the healthy virus carrier paradigm³.

POYNARD *et al.*²⁰ have studied liver fibrosis progression by analyzing large series of previously untreated HCV patients, with a

defined date of contamination and an average annual liver fibrosis progression rate calculated by means of a single liver biopsy specimen. Poynard's study originated a model suggesting a broad spectrum of progression and defining three groups of patients with fibrosis: those with slow progression fibrosis (cirrhosis over 50 years of age), moderate fibrosis (cirrhosis at 20 to 50 years of age), or rapid progression fibrosis (period of infection under 20 years). The literature shows that patients with normal ALT can represent the relatively slow fibrosis progression rate population^{12,13,16,19}.

There are currently six identified HCV genotypes and at least 80 subtypes⁷. In 1994²⁸, the nomenclature was standardized classifying HCV genotypes. The six different genotypes and the, at least, 80 genotypes have proven to vary geographically. Genotypic diversity affects numerous aspects of the disease for several reasons: in regard to HCV epidemiology because it varies geographically and also because determining the genotype is essential for studying the routes of infection; in regard to HCV pathogenicity because HCV strains have different levels of virulence, which may be responsible for co-infection with multiple genotypes, in regard to HCV treatment because different genotypes respond differently to medication.

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This study was carried out as part of a study protocol at the Medical Investigation Laboratory for Hepatitis (LIM-47) aimed at studying HCV and normal enzymes.

PATIENTS AND METHODS

Sixty-eight patients who were in accompaniment for chronic hepatitis C at the viral hepatitis clinic at the Infectious and Parasitary Diseases Department at the University of São Paulo School of Medicine were studied from January 1, 1997 to April 30, 2000.

Inclusion criteria were positivity for anti-HCV serology (immunoenzymatic method) by third and second generation ELISA, RNA-HCV detection by qualitative and/or quantitative PCR methods, a minimum of three normal (or 1.5-fold ULN) ALT levels over a 12-month period and a representative liver tissue specimen for histopathological analysis.

Routine biochemical tests were performed at the HC-FMUSP Central Laboratory according to the manufacture's standardized procedures (Diagnostics Corporation, Indianapolis, U.S.A.).

Medical residents at the Parasitary and Infectious Diseases Department carried out percutaneous liver biopsies preceded by ultrasound. Specimens were fixed in a 10%-solution of formalin and were subsequently stained with hematoxylin-eosin, Perls', Mason's trichrome and reticulin. All biopsy specimens were examined by a single pathologist according to the histopathologic and staging classification system of the Brazilian Society of Pathology¹⁰.

Genotyping was carried out on stored samples at the Laboratory for Hepatitis - LIM 47. 5'NC region was analyzed with PCR and genotyping tests.

Genotyping HCV

TRUGENE HCV 5'NC genotyping kit: a 244-bp fragment from the 5'NC of the HCV genome was amplified by RT-PCR using the COBAS AMPLICOR Hepatitis C test (Roche Molecular Systems, Inc., Branchburg, N.J.). The amplified products were purified with the QIAquick PCR purification kit (Quiagen, Inc., Valencia, CA) and sequenced with the TRUGENE HCV 5'NC Genotyping Kit according to the manufacturer instructions (Bayer Health Care Diagnostics, Berkeley, CA, USA). All HCV sequences were analyzed with the OpenGene DNA sequencer and Gene Object sequence analysis software (GL3.1.2)¹¹.

Serotyping analysis

Serotypes were determined by the Murex HCV serotyping 1-6 assay. This assay is used for the detection of type-specific NS4 derived epitopes, presented as branched peptides in a microliter 96-well format. Serotyping was performed according to the manufacturer's instructions. The results were scored as specific serotypes, multiple serotypes, no type-specific antibodies or non-reactive, according to the interpretation criteria established³⁰.

The Ethics Committees at both the Infectious and Parasitary Diseases

Department and at the Hospital das Clinicas have approved this study.

DATA ANALYSIS

The proportion of patients with ALT serum levels less than or equal to 1.5-fold ULN was calculated as a simple frequency, that is, the ratio between the number of patients with normal ALT levels or with up to 1.5-fold ULN and the total number of patients that received assistance at the clinic.

Considering the total number of patients chronically infected with HCV with normal ALT or ALT up to 1.5-fold ULN, assisted at the Viral Hepatitis Clinic at the Infectious Diseases and Parasitary Department at HC-FMUSP, during the period of January 1, 1997 and April 4, 2000 we calculated:

- The distribution of patients according to gender, age group, presence of steatosis, and genotype;
- The distribution of patients according to liver tissue structural alteration.

RESULTS

Prevalence of chronic hepatitis C and normal ALT (or 1.5 fold ULN) levels found was of 68/492 or 13.82% (CI 95%: 10.90%; 17.19%), of which 31 (45.6%) were men and 37 (54.4%) women. Patients' age varied from 18 to 71 years of age, with mean age being 38 +/- 13 years.

According to Figure 1, 73.5% of the 68 patients presented periportal activity score 0-1 and 26.5% of them periportal activity score 2-4. In terms of structural activity, in 85.3% of the cases patients scored 0-1 and in 14.7%, 2-4.

Eighteen out of sixty-eight or 26.5% of the patients had liver steatosis, of which 17 had mild steatosis and one moderate steatosis of the liver. Of the total number of patients, 50 (73.5%) had no liver steatosis at all.

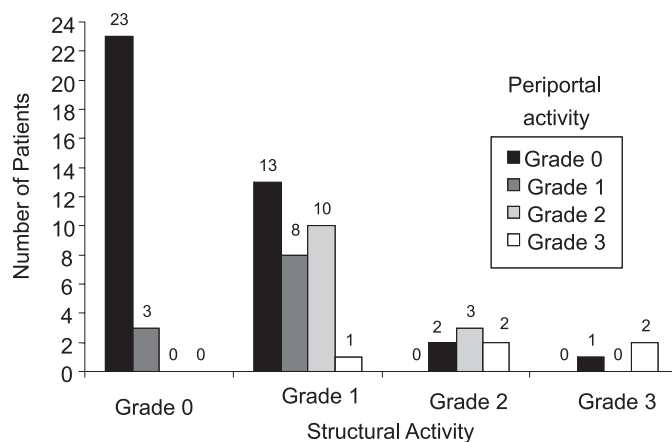


Fig. 1 - Distribution of patients with chronic hepatitis and normal ALT activity according periportal and structural activities.

Of 68 patients, 32 had chronic hepatitis. Of the latter, periportal activity (PPA) 0 and structural activity (SA) 0 were seen in three

patients; PPA 1 and SA 1, in eight; PPA 1 and SA 2, in two; PPA 1 and SA 3, in one; PPA 2 and SA 1, in ten; PPA 2 and SA 2, in three, PPA 3 and SA 1, in one; PPA 3 and SA 2, in two. Cirrhosis was not seen in any of the 32 patients.

Genotyping was carried out in 59 patients. 50 were genotype 1, of which seven were genotype 1, 25 were subtype 1b, 17 subtype 1a, and a mixed subtype 1a/1b was present in one patient. Four patients were genotype 2, of which one was genotype 2 and three patients were subtype 2b. Five patients were genotype 3a as shown in Table 1 below.

Table 1
Distribution of chronic hepatitis C patients and normal ALT activity according to genotypes

Genotypes	Number of patients	(%)
1	7	11.9
1a	17	28.8
1b	25	42.4
1a/1b	1	1.7
2	1	1.7
2b	3	5.0
3a	5	8.5
Total	59	100.0

DISCUSSION

Prevalence of hepatitis C with normal ALT varies from 7.5% to 46%^{1,15,16,17,19,22}. The 13.8% prevalence rate found is confirmed by the literature. All patients with normal ALT and ALT 1.5-fold ULN were included in this study and underwent liver biopsy. However, patients with only one or two ALT measurements in a year were not included and this probably contributed to a decreased prevalence rate.

The variation between the prevalence established in this study and prevalence rates in the literature is due to the fact that only patients with persistently normal ALT levels and patients with minimally raised ALT were included, to the variation in ALT levels obtained and to the lack of data on alcohol intake.

Therefore, this study included patients with normal ALT and patients with ALT not exceeding 1.5-fold ULN. Patients were selected according to the criteria in PUOTI *et al.*, which classify patients in two groups: the biochemically silent, with persistently normal ALT levels, and patients with minimally raise ALT¹⁸. 54.4% were women and 45.6% were men, although several studies indicate the prevalence of women in this population^{24,26,27}, this possibly suggests a protective effect of estrogens, which block the proliferation of stellate cells and fibrogenesis²¹. Another possibility is that, histologically, due to the different living styles adopted by men and women, especially concerning alcohol intake, which is a well-known factor in the pathological evolution process, the disease could be milder in women²¹.

Patients' age varied between 18 and 71 years of age, being the mean age 38 +/- 13 years, which corresponds to the expected age according to ALTER *et al.*² and FOCCACIA *et al.*⁸.

In regard to HCV genotypes, we found a prevalence of genotype 1 in 84.8% of the patients, followed by genotype 3 in 8.5% and by genotype 2 in 6.7%. These findings are similar to the findings in CAVALHEIRO *et al.*⁶ and BASSIT *et al.*³, who, in turn, do not determine ALT levels.

Genotype distribution found here is similar to that of Europe where there is also a prevalence of genotypes 1 and 3, and where dissemination probably occurred by means of blood transfusion.

PUOTI *et al.* compared a group of patients with elevated ALT and a group of patients with normal ALT and did not find an association in either group between HCV genotype and degree of liver lesion²⁴. This argument can be used against the predictive value of genotype.

Among the histological characteristics, steatosis has appeared as a fibrosis progression factor and is a common finding in chronic hepatitis C.

Serotyping for characterizing HCV type by immunoenzymatic method (ELISA) was performed in nine patients, because, although in at least one moment, these patients tested positive for RNA-HCV with PCR, in the samples examined retrospectively only results negatives for HCV were available.

Previous studies have also highlighted aspects of the relation between steatosis and HCV. Among said aspects, we can find high BMI and high levels of triglycerides¹⁴. In this study, information on BMI, triglyceride and glucose levels were not available in view of the fact that this is a retrospective study, and characteristics such as weight, height, triglyceride levels, were not always recorded on the patients' charts.

Cross-sectional studies indicate that liver steatosis may be a consequence of a cytopathic effect of hepatitis C virus, specially in patients infected with genotype 3. Steatosis is seen in approximately 30 to 70% of hepatitis C patients¹⁴.

In this study, steatosis was seen in 26.5% of the patients, of which only one had grade 2 steatosis; steatosis was not seen in 73.5%; and 10.5% were infected with genotype 3. These factors lead us to the hypothesis that fibrosis evolves at a lower rate in this population.

According to previous research, the data in this study show that the presence of HCV RNA is associated to the lesion, despite normal ALT activity. This suggests that the determination of chronic hepatitis, which is currently based on abnormal liver biochemical testing, should be reexamined since liver lesions are usually found in patients with normal ALT. Of 68 patients, in 52.9% of the cases biopsies showed liver reaction (reactional liver), which, according to the Brazilian Society of Pathology, reveals relatively smaller lesions than those considered to be hepatitis, but, even so, these lesions are not within the normality rates, because of the minimal aggressive action of the virus or of the nonspecific response of liver cells to other systemic procedures¹⁰.

The spectrum of liver lesions found in patients with chronic hepatitis C and normal ALT varies, but there is a general consensus in the literature that, in most cases, histological lesions are mild. The data in this study

are similar to those described in the literature^{1,9,18,24} according to which, although fibrosis takes place, it occurs less than in patients with elevated ALT, possibly because of the correlation between intensity of necroinflammatory activity and progression to fibrosis; so the histological lesions are milder pursuant to a recent study by FONTAINE *et al.*⁹.

According to world consensus, mild to severe hepatitis and compensated cirrhosis must be treated and the current criteria in effect established by the Brazilian Health Ministry in 2002⁴, stating that patients with PPA > 2 and/or SA > 1, which corresponds to 29% of the patients in this study, recommend antiviral treatment. ZEUZEM *et al.*³¹ in 2004 concluded that the efficacy and safety of peginterferon alfa-2a and ribavirin combination therapy in patients with chronic hepatitis C and persistently normal ALT levels are similar to that in patients with elevated ALT levels. The indication for treatment of hepatitis C can be evaluated independently from baseline ALT activity. This confirms that carrying out biopsies regardless of ALT levels, can contribute to tranquilizing patients with small lesions (who constitute the majority of the population), identifying severe liver lesions and selecting patients for treatment.

RESUMO

Pacientes com hepatite C crônica e transaminases normais

Hepatite C é uma doença de evolução progressiva. A maioria dos pacientes tem nível de ALT elevada e 25% apresentam níveis normais. Os com ALT normal geralmente são do gênero feminino e sem associação entre genótipo e gravidade de lesão hepática. O exame histopatológico mostra geralmente ausência de ou leve fibrose (cerca de 20% tem fibrose), embora cirrose já tenha sido relatada. Visando estimar a prevalência, características demográficas, genotípicas e anatomopatológicas em pacientes com ALT normal, realizamos um estudo de série com 68 casos com diagnóstico de hepatite C crônica. Os pacientes foram selecionados de janeiro de 1977 a abril de 2002. Encontrou-se uma prevalência de 13,8% (45,6% masculinos). A média de idade foi 39 +/- 13 anos. Predomínio de genótipo 1 (84,8%), seguido pelo 3 (8,5%) e 2 (6,7%). Encontramos fígado reacional em 52,9% das biópsias, atividade periportal de 0-1 em 85,3% e atividade periportal de 2-4 em 14,7%. Apresentaram atividade estrutural de 0 a 1 73,5% e 26,5% com atividade estrutural de 2 a 4, sendo que 29% da amostra apresentou APP ≥ 2 e AE > 1; 73,5% não apresentaram esteatose. Nossos dados reforçam a necessidade de biópsia em pacientes com hepatite C e níveis de ALT normais.

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