LIVER HISTOLOGY IN CO-INFECTION OF HEPATITIS C VIRUS (HCV) AND HEPATITIS G VIRUS (HGV)

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SUMMARY

As little is known about liver histology in the co-infection of hepatitis C virus (HCV) and hepatitis G virus (HGV), HGV RNA was investigated in 46 blood donors with hepatitis C, 22 of them with liver biopsy: co-infection HCV / HGV (n = 6) and HCV isolated infection (n = 16). Besides staging and grading of inflammation at portal, peri-portal and lobular areas (Brazilian Consensus), the fibrosis progression index was also calculated. All patients had no symptoms or signs of liver disease and prevalence of HGV / HCV co-infection was 15.2%. Most patients had mild liver disease and fibrosis progression index, calculated only in patients with known duration of infection, was 0.110 for co-infection and 0.130 for isolated HCV infection, characterizing these patients as “slow fibrosers”. No statistical differences could be found between the groups, although a lesser degree of inflammation was always present in co-infection. In conclusion co-infection HCV / HGV does not induce a more aggressive liver disease, supporting the hypothesis that HGV is not pathogenic.

KEYWORDS: Hepatitis C; Hepatitis G; Liver fibrosis; Inflammatory activity.

INTRODUCTION

Hepatitis C virus (HCV) and hepatitis G virus (HGV) are RNA viruses of the flaviviridae family that can be transmitted by blood transfusion and other parenteral and non-parenteral routes, with persistence of viremia1,2. The high prevalence of HCV infection and its morbidity is very well documented, as well as its relation to acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma3,16. As for HGV, a virus more recently described, there are few evidences of morbidity and many doubts about its etiologic role in liver diseases17,27.

The prevalence of HGV in blood donors is 1.5 to 1.7% in the United States, but among them only 50% have elevated ALT8. Many studies have detected the HGV-RNA in patients with chronic hepatitis and even some cryptogenic cirrhosis9, but the initial reports of HGV having an etiologic role in fulminant hepatitis were not confirmed in well conducted studies10.

Co-infection by HCV and HGV has been studied in chronic hepatitis submitted to interferon treatment11, in serum and liver of deceased drug users23, in association with cryoglobulinemia26, after bone marrow transplantation27 and also in terminal liver disease, before and after hepatic transplantation1. More recently, it was demonstrated that co-infection of HGV in HCV patients reduces mortality29, due to inhibition of HIV replication30. Histopathological variables, however, are seldom analyzed. In one of these studies histological severity of the underlying chronic hepatitis did not differ according to the HGV status, but hepatitis activity was moderately increased in patients with associated HIV infection28.

Taking in consideration the recent classifications of chronic hepatitis in which the stage of the disease is analyzed separately from necroinflammatory activity, the grading of which is evaluated in portal, peri-portal and lobular areas, it is worthwhile to investigate liver histology in co-infection by HCV and HGV. As the prevalence of hepatitis G is particularly high in Brazilian blood donors15, and hepatitis C infection is usually mild in these asymptomatic patients, this is an interesting group to investigate a possible synergism of both viruses in the production of liver damage.

PATIENTS AND METHODS

The subjects were 46 blood donors investigated due to hepatitis C positive tests. An epidemiological questionnaire was applied to detect the possible source of infection as well as its duration in years. Liver enzymes, namely ALT, GGT and other laboratorial tests were performed initially and every 2 or 3 months in the follow up in order to decide about liver biopsy and treatment. All the patients had no symptoms or signs of liver disease and were anti-HCV positive by ELISA II test confirmed by Immuno blotting test RIBA II. HGV-RNA was measured by nested polymerase chain reaction using primers from the NS5 region of the genome of HGV.

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Liver biopsy was indicated in patients with levels of ALT equal or greater than 1.5 times the upper normal limit, at two consecutive occasions, with more than 30 days interval. Biopsy specimens were processed for routine histopathological examination, using four different stains: Haematoxilin-Eosin, Masson’s trichrome, Perls for iron and impregnation of the reticulin framework by silver salts. Staging and grading of all specimens were semiquantitative from 0 to 4, in accordance with the criteria recently described by GAYOTTO et al.12 All this process was blind, that means without knowledge of HGV status. Fibrosis progression index (FPI) could be calculated only in those cases with a very well determined duration of infection.

FPI or fibrosis progression rate per year, indirectly estimated, is the ratio between the fibrosis stage in units (new classification of chronic hepatitis) and the estimated duration of infection in years. In this model, it is assumed that the patient has no fibrosis in the day of infection (F0) and that fibrosis progression rate is constant20. For example, in a patient with fibrosis stage 2 and 12 years duration of infection the fibrosis progression index is 0.166 units of fibrosis per year.

In order to compare the groups HCV+HGV and HCV only, the scores of the variables: staging, portal infiltrate, portal activity and lobular activity were submitted to variance analyzes by Wilcoxon score test. The values of p were obtained by the exact test of AGRESTI1. The procedure NPAR1WAY of the SAS system was utilized for the analysis of data.

RESULTS

HGV-RNA was detected in 7 of the 46 patients (15.2%). Liver biopsy was performed in 22 patients, six of them with co-infection HGV and HCV. Comparative demographic data of patients with co-infection or hepatitis C only, submitted to histopathological examination, is depicted in Table 1. Distribution of age and gender was the same between the groups, as well as serum levels of ALT and GGT.

Blood transfusion was the most frequent source of infection, present in one case with the HCV / HGV association and in 6 with HCV only. Other possible sources of infection in the HCV / HGV group were drug injections in one, tattooing in another and dentist manipulation in the fourth. In two patients no source of infection was found. Duration of infection was possible to be estimated in three patients of the HCV / HGV group and in seven of the control group. It has varied from 10 to 15 years in the HCV / HGV group, with a mean FPI of 0.110 and from 8 to 25 years in the hepatitis C group with a mean FPI of 0.130.

No patients in both groups reached grade 4 either in staging or inflammatory grading, as shown in Table 2. Most of the patients had mild liver disease and comparing the sum of grades 0 and 1 with the sum of grades 2 and 3, we have detected that inflammatory activity was usually lower in peri-portal areas and higher in lobular areas. When statistical analysis was applied the mean score values for inflammation varied from 9.41 to 9.83 for HVC+HGV and from 12.12 to 12.28 for HCV only. For staging (fibrosis) the mean score values were 11.25 for HCV only and 12.16 for HCV+HGV (Table 3). Although no statistical differences could be detected in terms of stage or activity between patients with co-infection compared to isolated HCV infection, a low degree of inflammation was constantly present in the HCV/HGV group.

DISCUSSION

The high prevalence of the association between HCV and HGV may be due to the fact that HGV is transmitted through the same routes as HCV, specially multiple blood transfusions, drug injection or other parenteral routes9. Nevertheless the prevalence of 15.2% is in keeping with that reported in literature, that has varied from 8.1%8, to 20%10 or 43%11, according to the epidemiology of the studied population. As we
Table 3  
Results of the 4 histological variables, according to Wilcoxon variance analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Sum of scores</th>
<th>Standard Deviation</th>
<th>Mean score</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staging</td>
<td>HCV+HGV</td>
<td>73.0</td>
<td>12.66</td>
<td>12.16</td>
<td>0.8628</td>
</tr>
<tr>
<td></td>
<td>HCV only</td>
<td>180.0</td>
<td>12.66</td>
<td>11.25</td>
<td>0.4415</td>
</tr>
<tr>
<td>Portal infiltrate</td>
<td>HCV+HGV</td>
<td>59.0</td>
<td>12.66</td>
<td>9.83</td>
<td>0.4291</td>
</tr>
<tr>
<td></td>
<td>HCV only</td>
<td>194.0</td>
<td>12.66</td>
<td>12.12</td>
<td>0.3503</td>
</tr>
<tr>
<td>Peri-portal activity</td>
<td>HCV+HGV</td>
<td>58.0</td>
<td>12.57</td>
<td>9.66</td>
<td>0.4291</td>
</tr>
<tr>
<td></td>
<td>HCV only</td>
<td>195.0</td>
<td>12.57</td>
<td>12.18</td>
<td>0.3503</td>
</tr>
<tr>
<td>Lobular activity</td>
<td>HCV+HGV</td>
<td>56.5</td>
<td>12.71</td>
<td>9.41</td>
<td>0.3503</td>
</tr>
<tr>
<td></td>
<td>HCV only</td>
<td>196.5</td>
<td>12.71</td>
<td>12.28</td>
<td>0.3503</td>
</tr>
</tbody>
</table>

have studied blood donors with mild forms of chronic hepatitis C, our prevalence is similar to the 10%, found in Brazilian healthy blood donors\(^5\).

Our patients had no symptoms but due to high levels of ALT, sometimes associated with elevated GGT, they were submitted to liver biopsy in order to decide about the treatment for hepatitis C. Evaluation of the histological variables is generally accepted as the main parameter for treatment indication and patients with grades 0 or 1 in all these parameters should not be submitted to the anti-viral treatment available so far\(^7\).

The main parameter to evaluate progression of liver disease is the development of fibrosis\(^7\). At histology, stage grade 2 means the presence of fibrous septa out of the portal space, whereas in grades 0 and 1 fibrosis is respectively absent and restricted to portal area. Considering this variable, our co-infected patients behaved exactly the same as those infected only by HCV.

Analyzing the fibrosis progression index, the more recent and accepted way to evaluate the progression of hepatitis C\(^21\) we have found similar results for co-infected and control groups. The index of 0.110 for co-infection and 0.130 for hepatitis C characterize our patients as “slow fibrosers”. In fact, the mean values found in the group of 1157 pre-treatment cases of POYNARD et al. was 0.252\(^20\).

Analyzes of the necroinflammatory activity according to the META VIR classification has the advantage of combining the scores in the three areas giving a single number to express this variable\(^4\). Nevertheless, as a disadvantage, we lose the possibility of interpreting and correctly appreciate the real value of inflammation in the three different areas. Using the Brazilian consensus for the classification of chronic hepatitis we have analyzed inflammation separately in portal, peri-portal (or interface) and lobular or parenchymal areas\(^2\). Although scores 2 and 3 were more frequently found in lobular than in peri-portal areas, in both groups of patients the statistical analysis did not show significant differences. Mean score values for HCV+HGV group were systematically lower than HCV alone, but also without statistical significance.

Similarly to what is described by other authors\(^5,11,22\) we could not detect a synergism between HCV and HGV and although not statistically significant, the necro-inflammatory activity was, in this small group of patients, a little lower in the co-infected patients. There are doubts about the pathogenicity of HGV\(^6,14,19,24\) and even if HGV is really a hepatropic virus with replication within liver cells. PESSOA et al.\(^18\) have shown that levels of HGV RNA in liver and serum were similar in patients with HGV infection alone compared with HGV/HCV co-infection. Differently from HCV, the median liver/serum levels of HGV RNA were less than unity, what is consistent with serum contamination of liver tissue, leading to the conclusion that liver is not the main site of HGV replication.

Although there are no clues for a protective function of HGV in hepatitis C, since it does not affect HCV replication, the recent findings of mortality reduction in patients co-infected with HIV + HGV, compared with those with HIV alone\(^29,30\), claim for a better evaluation of hepatic histology in patients co-infected with HGV. Our results, showing that co-infection does not induce a more aggressive liver disease, supports the hypothesis of no pathogenicity of HGV.

**RESUMO**

**Histologia hepática na co-infeccão do vírus da hepatite C (VHC) e vírus da hepatite G (VHG)**

As escassas informações sobre histologia hepática na co-infeccão do vírus da Hepatite C (VHC) e vírus da Hepatite G (VHG) nos levou a investigar o RNA-VHG em 46 doadores de sangue com hepatite C, dos quais 22 com biópsia hepática: co-infeccão VHC/VHG (n = 6) e infecção isolada do VHC (n = 16). Além do estadiamento e graduação da atividade inflamatória nas áreas portal, peri-portal e lobular, segundo o Consenso Brasileiro, calculamos também o índice de progressão da fibrose. Os pacientes estudados não apresentavam sintomas ou sinais físicos de doença hepática. A prevalência da co-infeccão VHC/VHG foi de 15,2%. A maior parte dos pacientes apresentava-se com lesão hepática discreta e o índice de progressão da fibrose, calculado apenas nos pacientes com duração conhecida da infecção, foi de 0,110 para os co-infetados e de 0,130 para aqueles com infecção isolada pelo VHC, caracterizando esses pacientes como “fibrosantes lentes”. Não foram encontradas diferenças estatísticas entre os grupos, apesar de menor grau de inflamação em todas as áreas analisadas, nos casos de co-infeccão. Em conclusão, a co-infeccão VHC / VHG não induz o surgimento de lesão hepática mais grave, favorecendo a hipótese de que o VHG não é patogênico.
REFERENCES


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