Hepatitis C Virus Infection Among Patients with Chronic Immune Thrombocytopenic Purpura in Northern India

Yogesh K Chawla**, Radha K Dhiman**

Departments of *Internal Medicine,**Hepatology, and †Histopathology,
Postgraduate Institute of Medical Education and Research, Chandigarh – 160012, India

Background: Hepatitis C virus (HCV) has been reported to be associated with the occurrence of autoimmune disorders, including immune thrombocytopenic purpura (ITP). This has suggested that HCV could be responsible for thrombocytopenia in these patients. This study was performed to estimate the frequency of HCV infection in patients of chronic ITP (cITP), and to find the frequency of thrombocytopenia in chronic HCV infection.

Materials: A total of 150 subjects were included in the study. Fifty consecutive adult patients with cITP (>6 months’ duration) and 50 age-matched patients with chronic HCV were included for comparison of platelet counts in two groups. Fifty age-matched healthy subjects were also included in the control group. All patients’ sera were tested for the presence or absence of HCV-RNA. Anti-HCV antibodies were tested in patients as well as in controls. Complete blood count and examination of peripheral blood smear were done followed by bone-marrow aspiration to confirm the diagnosis of ITP. Results: Three patients (6%) were tested positive for anti-HCV antibodies while no subject was positive in control group (P=0.24). The prevalence of severe thrombocytopenia (platelet counts <50,000/mL) was significantly higher in ITP patients compared with that in chronic HCV patients (P=0.0001). Thrombocytopenia occurred more frequently in patient with moderate to severe than mild stage of fibrosis (P=0.001). Conclusion: In conclusion, thrombocytopenia in ITP patients was not associated with HCV infection. The prevalence of thrombocytopenia was more common and more severe in ITP patients when compared with that in patients with chronic HCV. Thrombocytopenia in chronic HCV patients was related to the stage of fibrosis and to the duration of HCV infection. (J CLIN E XP H EPATOL 2011;1:68–72)

Keywords: Hepatitis C virus, hypersplenism, immune thrombocytopenic purpura, portal hypertension

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Address for correspondence: Radha K Dhiman, Professor, Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh – 160012, India
E-mail: rkpsdhiman@hotmail.com

Abbreviations: CI: confidence intervals; ELISA: enzyme-linked immunosorbent assay; HCV: hepatitis C virus; HIV: human immunodeficiency virus; ITP: immune thrombocytopenic purpura; PAIgG: platelet-associated immunoglobulin G; SLE: systemic lupus erythematosus

Immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by persistent thrombocytopenia due to antibodies against the platelet antigens, which result in platelet destruction in the reticuloendothelial system.1,2 Immune-mediated thrombocytopenia may be classified in terms of duration (acute vs chronic) or etiology (primary vs secondary). Acute form of ITP is generally seen in children and approximately 50% of these may have preceding viral illness. A chronic form of ITP (cITP) is generally a disease of adults which persists beyond 6 months. Secondary forms of ITP are commonly associated with systemic lupus erythematosus (SLE), antiphospholipid antibody syndrome, lymphoma, human immunodeficiency virus (HIV), and thyrotoxicosis.3 Immunologically mediated thrombocytopenia, mostly self-limiting, is also known to occur in association with bacterial and viral infections.4 Among the viral infections, HCV has been reported to be associated with the occurrence of autoimmune disorders, including ITP.

Infection with HCV is known to cause profound changes in the host immune response, including development of autoimmunity. Autoantibodies are commonly found in up to 40% cases of chronic HCV infection.5 It may be important to identify HCV-associated ITP because it may necessitate the control of viral replication along with the control of autoimmune phenomenon. Therefore, we conducted this study to estimate the frequency of HCV infection in patients with cITP, and to find the frequency of thrombocytopenia in chronic HCV infection.

MATERIALS AND METHODS

Patients
The Ethics Committee of Postgraduate Institute of Medical Education and Research (PGIMER), a tertiary-level health-care center in Chandigarh, India, approved the study. Fifty consecutive adults with cITP, attending the Hematology Clinic, PGIMER, Chandigarh, were enrolled in the study.
Patients were defined as cITP according to the guidelines set forth by the American Society of Hematology (ASH). Patients with >16 years and platelet count <150 × 10^3/mL lasting for >6 months were included in the study. All patients were diagnosed with cITP on the basis of bone-marrow examination which was suggestive of megakaryocytic thrombocytopenia. Patients with secondary forms of immune-mediated thrombocytopenia like hepatitis B virus infection, HIV, SLE, antiphospholipid antibody syndrome, chronic lymphocytic leukemia, myelodysplasia, liver disease with/without splenomegaly, hereditary thrombocytopenia, and those with a history of chronic drug intake such as acetaminophen, acyclovir, and carbamazepine were excluded from the study.

Fifty age-matched patients with chronic HCV attending the Liver Clinic of Department of Hepatology, PGIMER, Chandigarh, were included before starting antiviral therapy for comparison of platelet counts in two groups.

Controls
Fifty age-matched healthy subjects, who did not have any history suggestive of liver disease or any other illness in the past 2 weeks, were included in the control group.

Serology
All patients and controls were tested for the presence of anti-HCV in blood. All patients’ sera were also tested for the presence or absence of serum HCV-RNA. However, HCV-RNA testing was not done as a screening test in controls under the assumption that these were immunocompetent and should mount a serologic response against HCV infection, if it existed. A third-generation ELISA was used to detect antibodies against HCV using a cut-off OD value (0.450) calculated from negative and positive controls as described by the manufacturer (LG HCD 3.0 Plus; LG Chemical Ltd., Pharmaceutical division, Seoul, Korea). The test detected antibodies against three kinds of fusion proteins which are constituents of the HCV nucleocapsid; core 518, E1E2NS4, and NS5.

For the detection of hepatitis B surface antigen (HBsAg), the method used in our study was a direct immunoenzymatic assay of the “sandwich” type (Bioelisa HBsAg color; Biokit, SA, Barcelona, Spain).

HCV-RNA Detection
HCV-RNA was detected by the nested reverse transcriptase polymerase chain reaction. The primers were chosen from the conserved 5′ UTR region of the HCV genome.

The primer sequences that were used in this study were as follows:

- Outer sense primer (nucleotide position –297 to –278) 5′-CTG TGA GGA ACT ACT GTC TT-3′
- Outer antisense primer (position 8–36) 5′-ATA CTC GAG GTG CAC GGT CTA CGA GAC CT-3′
- Inner antisense primer (position –29 to –4) 5′-CAC TCT CGA GCA CCC TAT CAG GCA GT-3′
- Inner sense primer (position –279 to –260) 5′-TTC ACG CAG AAA GGC TCT AG-3′

Five age-matched patients with chronic HCV attending the Liver Clinic of Department of Hepatology, PGIMER, Chandigarh, were included before starting antiviral therapy for comparison of platelet counts in two groups.

Histopathology
Liver biopsies were performed in 28 patients. Staging of fibrosis in the liver biopsy tissue was done according to the scoring system of Ishak et al.7 The fibrosis stage ranged from stage 0 (no fibrosis) to stage 6 (cirrhosis of liver).

Statistical Analysis
Data are presented as mean and 95% confidence intervals (CI). Student’s t-test for unpaired data was used to compare groups when variables were normally distributed. Comparison between different groups was performed using Mann–Whitney test when variables were not normally distributed. A χ^2 test was used to compare differences in categorical variables; Yates correlation was applied when required. Spearman rank correlation was used to test for association between histological stage and platelet counts. A two-tailed P value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS software for windows, version 10 (SPSS Inc., Chicago, IL).

RESULTS
Clinical and demographic characteristics of the patients of cITP enrolled for the study are shown in Table 1. Mean duration of symptoms was 4.8 years (SD 4.7, range 1–20).

Table 1. Baseline characteristics and laboratory parameters of immune thrombocytopenic purpura and chronic hepatitis C patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>cITP (n = 50)</th>
<th>CHC (n = 50)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>36.2 (32.41–40.1)</td>
<td>38.6 (34.96–42.2)</td>
<td>0.605</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>17:33</td>
<td>43:07</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.8 (11.1–12.5)</td>
<td>13.6 (13.00–14.18)</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelet count (per μL)</td>
<td>53,234 (29,541–76,926)</td>
<td>207,540 (183,240–231,839)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Prothrombin time (sec)</td>
<td>12.1 (11.75–12.4)</td>
<td>13.4 (12.77–14.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>3</td>
<td>50</td>
<td>0.0001</td>
</tr>
<tr>
<td>HCV-RNA</td>
<td>2</td>
<td>50</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

cITP: chronic immune thrombocytopenic purpura; CHC: chronic hepatitis C. Data are expressed as mean and 95% confidence interval (CI).
Of these, 12 had bleeding manifestations (menorrhagia and epistaxis), remaining 38 had petechiae and purpura. The laboratory parameters of patients are presented in Table 2.

Three (6%) patients with cITP were tested positive for anti-HCV antibodies while no subject was positive in the control group. Among these three patients, two (4%) tested positive for RT-PCR. These two patients had received blood transfusion in the past 2 years. The remaining one anti-HCV positive patient was tested negative for HCV-RNA.

The prevalence of severe thrombocytopenia (platelet counts < 50,000/μL) was significantly higher in cITP patients compared with that in chronic HCV patients (P = 0.0001) (Table 3). Patients with chronic HCV and thrombocytopenia were older compared with those without thrombocytopenia (P = 0.015) (Table 4). Thrombocytopenia also occurred more frequently in patients with moderate to severe than mild stage of fibrosis (P = 0.001). These observations suggest that patients with longer duration of HCV infection and severe liver disease had more thrombocytopenia, as shown in Table 4. There is a progressive fall in platelet counts as the severity of disease progresses from mild to severe stage of fibrosis (r = −0.540, P = 0.004).

### Table 2: Laboratory parameters of chronic hepatitis C patients (n = 50).

<table>
<thead>
<tr>
<th>Variables</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes 1</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>Non-genotype 1 (genotypes 2, 3)</td>
<td>36 (72%)</td>
</tr>
<tr>
<td>Stage of fibrosis of liver biopsy&lt;sup&gt;7&lt;/sup&gt;</td>
<td>n (%)</td>
</tr>
<tr>
<td>Mild (Ishak stages 0–2)</td>
<td>16 (57.1%)</td>
</tr>
<tr>
<td>Moderate to severe (Ishak stages 3–6)</td>
<td>12 (42.9%)</td>
</tr>
<tr>
<td>Platelet counts (per μL)</td>
<td>223,733* (191,139–256,326)</td>
</tr>
<tr>
<td>Patients with mild fibrosis</td>
<td>139,428* (100,922–177,934)</td>
</tr>
</tbody>
</table>

*P = 0.015. Data are expressed as mean and 95% CI.

### Table 4: Age distribution and thrombocytopenia in hepatitis C virus patients.

<table>
<thead>
<tr>
<th>Characteristics of patients</th>
<th>Thrombocytopenic (n = 13)</th>
<th>Non-thrombocytopenic (n = 37)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr), mean (95% CI)</td>
<td>45.9 (37.3–54.6)</td>
<td>36.0 (32.2–39.8)</td>
<td>0.015</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>11:02</td>
<td>33:04</td>
<td>0.64</td>
</tr>
<tr>
<td>Genotype (n = 49)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (23.07%)</td>
<td>11 (29.72%)</td>
<td>0.73</td>
</tr>
<tr>
<td>2</td>
<td>2 (5.4%)</td>
<td>23 (62.16%)</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>10 (76.92%)</td>
<td>16 (43.24%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage (n = 28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild fibrosis</td>
<td>2 (15.38%)</td>
<td>16 (43.24%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Moderate to severe fibrosis</td>
<td>7 (53.84%)</td>
<td>3 (8.18%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

DISCUSSION

It is well known that the sero-prevalence of HCV infection in healthy subjects varies greatly from country to country. Because patients who develop a new infection with HCV are usually asymptomatic for many years, the true prevalence is difficult to estimate. Most studies have been observational in infected patients. Infection with HCV is known to cause profound changes in the host immune responses, including the development of autoimmunity. This autoimmune mechanism may have a role in the pathogenesis of hepatic damage as well as in the development of extra-hepatic complications of HCV. Of these extra-hepatic manifestations, HCV infection is known to be associated with ITP.4,6,8 Genotype 1a occurs with higher frequency and is more likely to be associated with thrombocytopenia.9 The main mechanism involved in immunopathogenesis has been suggested to be the activation of B cells following the binding of HCV to the CD-81 receptor on B cells, facilitating the production of antibody.10

It has been suggested that the binding of HCV to the platelets may induce the development of neoantigens on the platelet surface or it may alter the conformation of the platelet membrane glycoproteins, thereby contributing to autoimmune formation against target platelet glycoproteins.4 Immune complex-associated platelet clearance and reticuloendothelial destruction have also been proposed to contribute to thrombocytopenia in patients with chronic viral liver disease.11 According to Cines et al,12 splenic sequestration of platelets secondary to portal hypertension in chronic HCV may be the pathogenesis behind thrombocytopenia in HCV.

Elevated titers of PAIgG, which could represent immune complex-coated platelets, have been shown to
increase gradually as the severity of liver disease increases, suggesting that prolonged HCV infection causes marked immune system abnormalities. Antiplatelet–antibody titers were found to be high in most of the patients with chronic HCV infection. Aref et al observed that the platelet count was inversely correlated to the levels of platelet glycoprotein-specific antibodies, and significantly paralleled to spleen size. Platelet glycoprotein-specific antibodies represent a common mechanism inducing thrombocytopenia in patients with chronic HCV infection.

A high prevalence of thrombocytopenia has been reported in HCV-infected patients by some investigators. Thus, they have suggested that HCV could be responsible for ITP. A study by Nagamine et al showed that thrombocytopenia (<150 × 10^9/μL) was present in 41% of patients with chronic HCV infection. Elevated titers of PAIgG were observed in 88.1% of patients with chronic HCV. Some studies have shown that treatment of HCV infection with interferon has resulted in improvement of platelet count. Sakuraya et al observed that in the case of HCV associated with ITP, the response rate of ITP to prednisolone quoted by the author may be due to continuous disturbance of immune system by HCV infection which reduces the steroid efficacy.

Weksler et al from the USA reviewed literature to find out the various causes of thrombocytopenia in chronic HCV infection. In patients with untreated HCV infection, both prevalence and severity of thrombocytopenia are directly proportional to the extent of the disease, usually becoming clinically relevant when patients develop extensive fibrosis and/or cirrhosis. Pathogenetic mechanisms include hypersplenism secondary to portal hypertension, bone-marrow suppression resulting from either HCV itself or interferon treatment, aberrations of the immune system resulting in the formation of antiplatelet antibodies and/or immune complexes that bind to platelets and facilitate their premature clearance and thrombopoietin deficiency secondary to liver dysfunction. According to Chiao et al, HCV was associated with elevated risks for ITP (HR, 1.8; 95% CI, 1.4–2.3). The ITP incidence was increased among both untreated and treated HCV-infected persons (HR, 1.7; 95% CI, 1.3–2.2 and HR, 2.4; 95% CI, 1.5–3.7, respectively).

Moreover, HCV infection can also be associated with chronic thrombocytopenia, even in the absence of overt liver disease. While HCV-related thrombocytopenia is typically less severe than primary cITP, affected patients are at greater risk of major bleeding. Sustained suppression of HCV virus with interferon–ribavirin combination therapy can improve platelet counts. Screening for HCV infection should be considered in patients with ITP with risk factors for infection, from regions with high rates of infection or in patients with unexplained mild elevations of liver enzymes.

In this study, we did not find significant association between HCV infection and ITP. The prevalence of HCV positivity in the ITP group was 6%, which though higher than general prevalence (<1%) was statistically insignificant when compared with control group 0%. This may be a true nonsignificance or may be related to a small sample size. Three patients who were tested positive were in the mean age group of 56.3 years (range 44–75 years), mean duration of presentation was 2 years, and mean platelet count was 70,000/μL (range 12,000–93,000/μL). The sero-prevalence of HCV infection in adult ITP patients was not statistically different than that of controls, indicating that HCV is not involved in causation of ITP. Moreover, ITP patients who tested positive to anti-HCV/HCV-RNA might have acquired infection after the diagnosis of ITP, possibly due to blood transfusion.

In 50 cases with chronic HCV infection, thrombocytopenia (platelet counts, <150 × 10^9/μL) was seen in 26%. The occurrence of severe thrombocytopenia (platelet counts, <50,000/μL) was high in cITP patients as compared with CHC group. Our study also demonstrates that chronic HCV infection is not associated with ITP, while thrombocytopenia in chronic HCV patients is mainly related to the severity (fibrosis stage) of liver disease.

In conclusion, this study has not shown the association of HCV infection with ITP in northern India, and thrombocytopenia in HCV infection is related to the stage of fibrosis of liver and to the duration of liver disease.

CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES