Clinical Relevance of Cryoglobulinaemia and Extrahepatic Neurocutaneous Manifestations of Chronic Hepatitis C

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Abstract

Objective
To examine the prevalence of cryoglobulinaemia in patients with chronic hepatitis C infection and its relation to extrahepatic neuro-cutaneous manifestations.

Methods
Forty patients (26 males & 14 females), with clinical, laboratory and histologically established chronic hepatitis C infection, with a mean age of 37.5 years, were submitted to clinical examination, dermatological and neurological evaluation. Neuroimaging as well as neurophysiological evaluation, laboratory assessment including liver function tests, serum cryoprecipitate immunoelectrophoresis, and revision of histopathological findings were performed.

Results
A high prevalence of cryoglobulenemia: 62.5% in patients with chronic hepatitis C infection, the presenting symptoms were fatigue (67.5%), arthralgia (32.5%), paresthesia (30%) and pruritus (25%); however, there were no statistically significant difference between cryo +ve versus cryo -ve patients except for pruritis, and face pigmentation. Skin manifestations including face pigmentation (42.5%), leukocytoclastic vasculitis (22.5%), porphyria cutanea tarda (20%), lichen planus (17.5%), acral necrolytic erythema (15%) and vitiligo (15%). Neurological manifestations; symptomatic neuropathy in 10%, neuropathic changes in 30% and electroencephalographic changes in 22.5%. These cutaneous and neurological manifestations were significantly associated with the presence of cryoglobulinaemia.

Conclusion
Our findings support an association between cryoglobulinaemia and extrahepatic neurocutaneous manifestations of hepatitis C infection. The presence of all these manifestations in the appropriate clinical setting should suggest the presence of hepatitis C infection.

Key Words: Chronic hepatitis C, cryoglobulinaemia, extrahepatic neurocutaneous manifestations


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Introduction

Nowadays, there is an increasing awareness of a variety of extrahepatic syndromes that seem to be associated with HCV infection. Pascual et al. reported two patients with chronic HCV infection and essential mixed cryoglobulinaemia (EMC). Since then, a considerable number of extrahepatic syndromes has been reported as described by Nocente et al.

Cryoglobulinaemia (Cg) is a condition characterized by the presence of serum proteins that reversibly precipitate in cold and are classified on the basis of their immunoglobulin composition according to Brouet et al. The clinical manifestations of mixed cryoglobulinaemia (MC) range from asymptomatic, mild vasculitis with palpable purpura, arthralgia and fatigue, to severe vasculitis with skin necrosis, involvement of the kidneys, peripheral nerves, and central nervous system. Fifty percent of patients with chronic HCV, show detectable cryoglobulinaemia, even though most of them do not show cryoglobulinaemia related symptom. Factors involved in the production of Cg in HCV infected patients are unknown; most likely the presence of HCV in the cells of the immune system and/or chronic stimulation of the immune response by HCV. Also, genetic factors may be involved in the pathogenesis of Cg. Little is known about the clinical significance of Cg in the course of chronic HCV.

Neurological manifestations of HCV have been less well characterized and reported, including peripheral nerve vasculitic neuropathy, and also intracranial vasculopathy. Peri...
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Schistosomiasis, hepatitis B, autoimmune or drug-induced hepatitis.

3. The presence of other causes of cryoglobulinaemia including HIV, malignancy, or other chronic diseases.

4. Previous antiviral therapy in the patient population before their assessment. Patients were selected from patients who were evaluated for antiviral therapy at the Clinic of Gastroenterology and Hepatology of Specialized Medical Hospital Mansoura University during January 2004 to January 2006. Patients were submitted to clinical examination, dermatological and neurological evaluation, neuroimaging study (CT and MRI brain) as well as neurophysiological evaluation, laboratory assessment including liver function tests, serum cryoprecipitate immune-electrophoresis, antinuclear antibody, rheumatoid factor as well as abdominal ultrasound and revision of histopathological findings.

Detection of Cryoglobulins

The thermolability of cryoglobulins necessitated that the blood samples be collected at 37°C. Syringes and collection tubes were at 37°C at the time of blood collection and maintained at 37°C until clotting is completed. Tubes for collection were not anticoagulated, since the use of plasma may result in the development of cold-precipitable fibrinogen (cryofibrinogen) or heparin-precipitable protein. Blood Sampling 10mL of blood in a warm syringe (37°C) were collected.

Analysis Process:

A white precipitate (cryoglobulin) appeared in the serum after 24-72 hours of storage at 4°C. The serum was tested for the reversibility of the cryoprecipitate by re-warming an aliquot at 37°C for 24 hours. The cryocrit was estimated by measuring the height of the column of precipitated protein relative to the height of the serum column.

Liver histology was evaluated according to the standard international criteria using Metavir Score (Table 1).

Statistical Methods

Frequency, mean, standard deviation and standard error of mean were used to describe data. Mann-Whitney \( \mu \) test was used to test for significance of difference in quantitative variables between each two groups. Chi-square test was used to test for association between groups and clinical categorical data. P value was considered significant if \( < 0.05 \).

These tests were run on an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS) for windows 7.5 (SPSS Inc, Chicago, IL, USA). The study protocol was approved by human ethics committee of Specialized Medical Hospital Mansoura University.

As regard to laboratory findings including liver function tests (SGPT, serum bilirubin and prothrombin time), urine analysis (for proteinuria), serum creatinine, ANA, RF and quantitative PCR for HCV-RNA, there were no statistically significant difference between cryo +ve and cryo –ve patients except for proteinuria. Concerning the liver histopathological findings (evaluated according to Metavir score) there were no statistically significant difference in activity score between cryo +ve versus cryo –ve patients.

Results

The results are shown in Tables 2-5.

Table 1: Metavir Score A0-3 (Activity)  F0-4 (Fibrosis) \(^{18}\)

<table>
<thead>
<tr>
<th>Activity</th>
<th>A0 (None)</th>
<th>A1 (Mild)</th>
<th>A2 (Moderate)</th>
<th>A3 (Severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis</td>
<td>F0</td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
</tr>
</tbody>
</table>

Activity (A): An algorithm of both piecemeal and parenchymal necrosis

Fibrosis (F): Non, F1: Portal tract expansion by fibrosis, F2: <50% bridging fibrosis, F3: >50% bridging fibrosis including incomplete cirrhosis, F4: Established cirrhosis

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Table 2 shows the demographic details, patient clinical characteristics, skin and the neurological manifestations of the studied patients. Among 40 patients with chronic hepatitis C, there were 26 male (65%) and 14 female (35%). The main age of the patients was 37.5 years. The most common symptom was fatigue 27 cases (67.5%). Leukocytoclastic vasculitis was the main skin lesion 9 cases (22.5%) followed by porphyria cutanea tarda 8 cases (20%) and Lichen planus 7 cases (17.5%). Symptomatic neuropathy was only found in 4 cases (10%) while in 12 cases (30%) neuropathy was detected by nerve conduction velocity and EMG. EEG changes were detected in 9 cases (22.5%).

Discussion

The present study demonstrated a high prevalence of Cg: 62.5% in patients with chronic HCV infection. Different studies showed wide variation from 19% - 57%19-21. These discrepancies may be caused by different Cg detection methods and a strong regional differences.

Table 2 shows that many skin lesions were detected in the studied patients; including face pigmentation (42.5%), leukocytoclastic vasculitis (LCV) (22.5%), porphyria cutanea tarda (PCT) (20%), lichen planus (17.5%), acral necrolytic erythema (15%) and vitiligo (15%). Arthur et al16 found that cutaneous symptoms or findings relevant to HCV infection manifest in 20 – 40% of patients presenting to dermatologists and in a significant percentage (15 – 20%) of general patients.

Neurological manifestations, including symptomatic neuropathy in 10%, neuropathic changes (demyelinating neuropathy) detected by nerve conduction velocity (NCV) and electromyography (EMG) in 30%, also electroencephalographic changes in 22.5%. Our results are in accordance with that of Zaltron et al10, Ripault et al13, Bonetti et al22, and Arthur et
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These cutaneous and neurological manifestations detected in the studied patients were significantly associated with the presence of cryoglobulinaemia.

Table 3: Comparison of clinical characteristics in cryo +ve versus cryo –ve patients

<table>
<thead>
<tr>
<th>Features</th>
<th>Cryoglobulinaemia</th>
<th>Chi square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ve</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>9</td>
<td>18</td>
<td>0.615</td>
</tr>
<tr>
<td>Parasthesia</td>
<td>3</td>
<td>9</td>
<td>1.143</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>4</td>
<td>9</td>
<td>0.372</td>
</tr>
<tr>
<td>Pruritis</td>
<td>0</td>
<td>10</td>
<td>8.000</td>
</tr>
<tr>
<td>Edema</td>
<td>0</td>
<td>2</td>
<td>1.263</td>
</tr>
<tr>
<td>Face Pigmentation</td>
<td>1</td>
<td>16</td>
<td>12.610</td>
</tr>
<tr>
<td>Jaundice</td>
<td>5</td>
<td>7</td>
<td>0.127</td>
</tr>
<tr>
<td>BMI:</td>
<td>low</td>
<td>average</td>
<td>over</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>10.743</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows comparison of clinical characteristics in cryo+ve { 25/40 (62.5%) } versus Cryo –ve patients { 15/40 (32.5%) }. Cryoglobulinaemia was detected in 25 cases (62.5%). There were no statistically significant difference in clinical characteristics between Cryo –ve and Cryo +ve cases apart from pruritis, face pigmentation and BMI.

Table 4: Relation of skin manifestations in patients with and without cryoglobulinemia

<table>
<thead>
<tr>
<th>Features</th>
<th>Cryoglobulinemia</th>
<th>Chi square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ve</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Leucocytoclastic vasculitis</td>
<td>0</td>
<td>9</td>
<td>6.968</td>
</tr>
<tr>
<td>Spider nevai</td>
<td>1</td>
<td>9</td>
<td>4.302</td>
</tr>
<tr>
<td>Palmar Erythema</td>
<td>0</td>
<td>9</td>
<td>6.968</td>
</tr>
<tr>
<td>PCT</td>
<td>0</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Lichen Planus</td>
<td>0</td>
<td>7</td>
<td>5.091</td>
</tr>
<tr>
<td>Acral NE</td>
<td>0</td>
<td>6</td>
<td>4.235</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>0</td>
<td>6</td>
<td>4.235</td>
</tr>
</tbody>
</table>

Table 4 shows the relation of skin manifestations in cryoglobulinemia +ve versus Cryoglobulinemia –ve patients. Skin lesions including leucocytoclastic vasculitis, spider nevai, palmer erythema, PCT, Lichen Planus, Acral NE & Vitiligo showed statistically significant difference between cryo +ve and cryo -ve cases.
Table 5: Comparison of neurologic findings (clinical and neurophysiological) in Cryo+ve versus Cryo–ve patients

<table>
<thead>
<tr>
<th>Features</th>
<th>Cryoglobulinemia</th>
<th>Chi square</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ve</td>
<td>+ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic neuropathy</td>
<td>0</td>
<td>21</td>
<td>2.667</td>
<td>0.102</td>
</tr>
<tr>
<td>Myopathy</td>
<td>0</td>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fits</td>
<td>0</td>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EMG: Neuopathic</td>
<td>0</td>
<td>12</td>
<td>10.286</td>
<td>0.001</td>
</tr>
<tr>
<td>NCV: Delayed</td>
<td>0</td>
<td>12</td>
<td>10.286</td>
<td>0.001</td>
</tr>
<tr>
<td>EEG: Abnormal</td>
<td>0</td>
<td>9</td>
<td>6.968</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Table 5 showed comparison of neurologic findings (clinical and neurophysiological) in Cryo+ve versus Cryo–ve patients. There were no statistically significant difference between Cryo+ve and Cryo–ve patients for symptomatic neuropathy while there were statistically significant difference for abnormalities detected by electrophysiological studies (EMG, NCV & EEG).

Origgi et al\textsuperscript{14} stated that cryoglobulinaemia may be one of the mechanisms (vasculitis associated with cryoglobulinaemia and or multiple ischaemic changes) underlying cutaneous and neurological manifestations associating HCV infection. Chronic sensory polyneuropathy was the most frequent neurological manifestation, and this was the same as findings by Nemni et al\textsuperscript{23} who found that the prevalence of polyneuropathy was significantly higher in cg +ve versus cg -ve patients, however, Paolelli et al\textsuperscript{24} and Lidove et al\textsuperscript{25} reported some HCV+ve patients with polyneuropathy and persistent negativity for cryoglobulins.

Proteinuria was present in 22.5% of cryo positive patients in the current study. Valentina et al\textsuperscript{26} found that the presence of proteinuria correlated with very high cryocrit level. Other biochemical parameters and Quantitative PCR in the studied patients, showed no statistically significant difference between cryo positive and cryo negative patients. These were in accordance with Horcayada et al\textsuperscript{31} and valentina et al\textsuperscript{26}. Liver histopathology of the studied patients according to METAVIR score (Activity)A0-3 (Fibrosis) F0-4 showed no statistically significant difference between cryo positive versus cryo negative patients. These observations are in accordance with the data reported by Valenta et al\textsuperscript{26} and Schmidt et al\textsuperscript{21} who also found no correlation between the amount of Cg and histological activity. Sansonno and Dammacco\textsuperscript{27} reported that HCV-infected people with cryoglobulinemic vasculitis frequently show minimal liver damage and normal or slightly increased levels of serum aminotransferases. Several authors have reported a higher prevalence of Cg in patients with HCV-induced liver cirrhosis than in chronic hepatitis C without cirrhosis; however, this observation has not been confirmed by others Lunel et al\textsuperscript{28}. Also, Wong et al\textsuperscript{19} reported no differences in the stage of fibrosis between patients with and without cryoglobulinaemia.

**Conclusion**

Our findings confirmed a clear association between cryoglobulinaemia and extrahepatic neurocutaneous manifestations of HCV infection. The presence of these manifestations in the appropriate clinical setting should suggest the presence of HCV infection and HCV antibodies should be
tested and, if positive, HCV-RNA is indicated and if there is any evidence of an etiological association of replicative HCV infection and these extrahepatic manifestations, antiviral treatment should be considered.

We are in need to extend this work to study Hepatitis C virus induced cryoglobulinaemia quantitatively; essential mixed cryoglobulinaemia (EMC), and to assess if it has any role in HCV-liver disease progression.

References