Chronic Hepatitis C Virus (HCV)-associated Cryoglobulinemia and its possible impact on the skin in Egyptian Patients

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Abstract

Background:
Chronic hepatitis C virus (HCV) infection may have extremely variable clinical consequences and is more than just a liver disease; it has been associated with numerous extra-hepatic manifestations (EHM). According to various international studies Mixed Cryoglobulinemia (MC) was found to be the most common EHM, however its local prevalence in Egyptian HCV patients was not clearly studied. The aim of our study was to investigate the frequency of cryoglobulinaemia in sera of chronic HCV patients and their association with clinical symptoms especially, vasculitis.

Method:
One hundred patients with chronic HCV infection attending the outpatient clinic of the National Hepatology and Tropical Medicine Research Institute were interviewed. Patients with decompensated liver disease, on interferon therapy, having end-stage renal disease or coexisting viral infection like hepatitis B surface antigen positive patients were all excluded from the research. All patients were subjected to general and dermatological examination for liver affection signs; cryoglobulinemia related clinical manifestations and/or associated dermatoses. Cryoglobulins, CBC, LFT. AFP, ALP, KFT, ANA and RF were assessed.

Results:
Overall 15% of 100 patients were positive for presence of cryoglobulins in their sera. We found a relatively high incidence of clinical symptoms commonly accompanying cryoglobulinemic cases in the form of Purpura, Arthralgia, Generalized weakness, Peripheral Neuropathy and Reynaud’s phenomenon with prevalence of 26.67%, 46.67%, 53.33%, 40% and 6.67% respectively. Our data also demonstrated that 46.67% (7) of our 15 cryopositive patients had concomitant skin manifestations in the form of Pruritus 40% (6) and Vasculitis 26.67% (4) (P=0.004) which was significant in comparison with prevalence of vasculitis in all patients 4.7% (4 patients). Generalized weakness and fatigue, which is the most prevalent symptoms related to Chronic Hepatitis C (CHC) patients whether positive or negative for cryoglobulinemia, were present in 52% of all patients. Other associated dermatological diseases in all patients were Lichen Planus, Psoriasis, Urticaria, Necrolytic Acral Erythema (NAE) and Vitiligo with prevalence of (1%), (1%), (5%), (1%) and (2%) respectively. The most common dermatological disease was pruritus 36% (36) of all patients and 40% (6) in cryopositive patients. Regarding liver condition there was no significant correlation between presence of cryoglobulins and biochemical parameters. However, we found a significant correlation between presence of cryoglobulinemia and presence of ANA in sera of HCV patients with incidence of 40%) in
comparison to 4.7% in cryonegative patients respectively while there is no significant correlation as regard RF (40% in cryopositive & 55.3% in cryonegative patients). Among all patients 10% (10) were ANA positive and 53% (53) were RF positive. We also found that 26.67% (4) of our cryopositive patients were associated with vasculitic skin lesions. For each patient a skin biopsy was taken from the lesional area and tissue (Insitu) PCR was done to detect the presence of HCV RNA within it. Biopsy results revealed that 3 biopsies were positive and 1 biopsy was negative for HCV RNA by tissue PCR.

**Conclusion:**
The prevalence of cryoglobulinaemia in Egypt may be lower than other areas. In HCV patient complaining from generalized weakness, arthralgia, purpuric skin lesions, peripheral neuropathy, Reynaud’s phenomenon or renal troubles, serum cryoglobulins presence should be searched for.

**Introduction**
Chronic hepatitis C virus (HCV) infection affects more than 170 million persons worldwide and responsible for the development of liver cirrhosis in many cases (Wohnsland et al., 2007).

HCV infection is considered the most common etiology of chronic liver disease in Egypt, where prevalence of antibodies to HCV (anti-HCV) is approximately 10-fold greater than in the United States and Europe (Strickland et al., 2002). The prevalence among the general Egyptian population is approximately 13% (Frank et al., 2000; Habib et al., 2000).

Chronic HCV infection has been associated with numerous extrahepatic manifestations; these manifestations can involve multiple organ system, including renal, dermatologic, hematologic and rheumatologic systems (Gumber et al., 1995; El-serag et al., 2002).

The most common EHM is Mixed Cryoglobulinemia, cryoglobulins are found in 50% of chronic HCV infection patients. Cryoglobulins are found to be 11 times more frequent in a veterans HCV cohort group than their respective control population (El-serag et al., 2002). Cryoglobulins are immunoglobulins that precipitate at a temperature below 37°C. Mixed cryoglobulinemia is a chronic immune complex-mediated disease strongly associated with hepatitis C virus (HCV) infection. It is a vasculitis of small and medium-sized arteries and veins, due to the deposition of complexes of antigen, cryoglobulin and complement in the vessel walls.

The main clinical features of mixed cryoglobulinemia vasculitis include the triad of palpable purpura, arthralgias, and weakness, and other pathological conditions such as glomerulonephritis, peripheral neuropathy, skin ulcers, and widespread vasculitis (Iannuzzella et al., 2010). Although circulating cryoglobulins are not always related to the presence of symptomatology, nearly half of the patients with cryoglobulinemia present clinical manifestations (Ferri et al., 2002). Severe symptoms from cryoglobulinemia appear to respond to interferon treatment, but relapse occurs frequently once treatment is discontinued (Lunel et al., 1999).

**Aim of The Work**
Purpose of the study is to assess the presence of cryoglobulinemic immunoglobulins in serum of chronic HCV patients and its possible impact on the skin as a manifested Cryoglobulinemic Vasculitis.

**Patients and Methods**
The study included one hundred Egyptian persons, had chronic hepatitis C
virus (HCV) infection [proved by +ve anti-HCV antibodies and +ve HCV RNA by Quantitative Polymerase Chain Reaction (PCR) tests], were collected from the outpatient clinic at The National Hepatology and Tropical Medicine Research Institute (NHTMRI). Patients with decompensated liver disease, on interferon therapy, having end-stage renal disease or coexisting viral infection like hepatitis B surface antigen positive or HIV patients, patients with either hematological disorder, cancer and autoimmune systemic diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), were all excluded from the study.

All patients were subjected to the following standard protocol:

Full medical history, Full clinical examination and Laboratory investigations including: CBC, Liver function tests, Renal function tests, Plasma alpha fetoprotein (AFP), Serum ANA and RF, HBsAg, HBeAb and Anti HIV antibodies (ELISA), Anti HCV antibodies (ELISA), Quantitative HCV RNA by real time PCR test (f or HCV +ve ELISA), Qualitative Cryoglobulin precipitation test, abdominal Ultrasonography (U/S), and Skin biopsies: From patients with vasculitis (one biopsy from area just near the vasculitic lesions and the second one is from non-lesional areas in the same anatomic site), for detection of HCV-RNA by Polymerase chain reaction (HCV-RNA PCR) insitu.

Statistical analysis:
The clinical and laboratory data were recorded on an investigative report form. These data were analyzed using the mean, standard error, student t-test and chi-square by SPSS 13.

Results
The results are shown in tables 1-4

The data of this study demonstrated presence of cryoglobulins in 15/100 patients with an incidence of 15% of all patients (Figure 1).

In this study the 15 cryopositive patients were found to have a relatively high incidence of clinical symptoms which are commonly accompanying cryoglobulinemic cases in the form of Purpura, Arthralgia, Generalized weakness, Peripheral Neuropathy and Reynaud’s phenomenon with prevalence of 26.7%, 46.66% , 53.33%, 40% and 6.67% respectively (Table no.1). It was also demonstrated that 46.67% (7/15) of the cryopositive patients had concomitant skin manifestations in the form of pruritus 40% (6/15) and vasculitis 26.67% (4/15). None of the cryopositive patients had NAE (Table no.2).

Other associated dermatological diseases in all patients were Lichen Planus, Psoriasis, Urticaria, Necrolytic Acral Erythema (NAE) and Vitiligo with prevalence of (1%), (1%), (5%), (1%) and (2%) respectively. The most common dermatological disease was pruritus in 36 (36%) of all patients and in 6 (40%) of cryopositive patients (Table no.2).

Regarding ANA and RF there was a significant correlation between presence of cryoglobulinemia and presence of ANA in sera of HCV patients (with incidence of 40%) in comparison to 4.7% in cryonegative patients while it is insignificant as regard the presence of RF (40% in cryopositive &55.3% in cryonegative patients) (Table no.1).

HCV RNA was detected in skin biopsy of 3/4 (75%) of patients with cryopositive purpuric cutaneous lesions clinically diagnosed as vasculitis. However, HCV RNA was not detected in normal skin of same patients (Table no.3). High viral load was detected in 11/15 (73.33%) of the cryopositive patients and in only 29/85 (34.12%) of the cryonegative patients (Table no.4).
**Figure (1):** Shows the incidence of cryoglobulinemia in the patients of the study:

- Cryopositive: 15%
- Cryonegative: 85%

**Table (1):** Shows the distribution of important clinical findings, ANA and RF in cryo+ve patients in comparison with cryo-ve patients:

<table>
<thead>
<tr>
<th>Related clinical manifestations + ANA and RF</th>
<th>Cryoglobulins</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cryo +ve (% from all cryo+ve)</td>
<td>Cryo -ve (% from all cryo-ve)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>7 (46.67%)</td>
<td>34 (40%)</td>
</tr>
<tr>
<td>Purpura</td>
<td>4 (26.67%)</td>
<td>4 (4.7%)</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>6 (40%)</td>
<td>17 (20%)</td>
</tr>
<tr>
<td>Generalized weakness</td>
<td>8 (53.33%)</td>
<td>44 (51.76%)</td>
</tr>
<tr>
<td>Reynaud’s phenomenon</td>
<td>1 (6.67%)</td>
<td>2 (2.3%)</td>
</tr>
<tr>
<td>ANA</td>
<td>6 (40%)</td>
<td>4 (4.7%)</td>
</tr>
<tr>
<td>RF</td>
<td>6 (40%)</td>
<td>47 (55.29%)</td>
</tr>
</tbody>
</table>

*significant at P-value<0.05
Table (2): Shows prevalence of dermatological diseases among Cryo+ve patients in comparison with cryo-ve patients:

<table>
<thead>
<tr>
<th>Other skin disease</th>
<th>Cryoglobulins</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cryo +ve (% from all cryo+ve)</td>
<td>Cryo -ve (% from all cryo-ve)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>6 (40%)</td>
<td>30 (35.29%)</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>0</td>
<td>1 (1.17%)</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>0</td>
<td>1 (1.17%)</td>
</tr>
<tr>
<td>Necrolytic Acral Erythema</td>
<td>0</td>
<td>1 (1.17%)</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>0</td>
<td>2 (2.35%)</td>
</tr>
<tr>
<td>Urticaria</td>
<td>0</td>
<td>5 (5.88%)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>4 (26.67%)</td>
<td>4 (4.71%)</td>
</tr>
</tbody>
</table>

*significant at P-value<0.05

Table (3): Shows the presence of HCV-RNA Insitu in the biopsies of vasculitic skin lesions among cryo+ve patients:

<table>
<thead>
<tr>
<th>Skin biopsy</th>
<th>Cryo +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculitis</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>HCV-RNA-PCR Insitu (+ve)</td>
<td>3 (75%)</td>
</tr>
</tbody>
</table>
Table (4): Shows the presence of high viremia among cryo+ve patients in comparison with cryo-ve patients:

<table>
<thead>
<tr>
<th>Cryoglobulins</th>
<th>Patients with high viral load (% of high viremia)</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (15 patients)</td>
<td>11 patients (73.33%)</td>
<td>6.618</td>
<td>0.01* (significant)</td>
</tr>
<tr>
<td>Negative (85 patients)</td>
<td>29 patients (34.12%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*significant at P-value<0.05

Discussion

Chronic hepatitis C virus (HCV) is emerging as a serious worldwide problem, being the second most common chronic viral infection in the world with a global prevalence of about 3% (about 180 million people)(Craxo et al., 2008). Egypt has the highest prevalence of HCV infection in the world, 8-30% of the general population varying according to age group and according to whether in urban or rural areas; this is ten times greater than any other country in the world (Strickland, 2006, El-Hawary et al., 2007 and Marzouk et al., 2007). HCV infection may have extremely variable clinical consequences and is more than just a liver disease, it has been associated with numerous extra hepatic manifestations (EHM), and these manifestations can involve multiple organ system, including renal, dermatologic, hematologic and rheumatologic system (Galossi et al., 2007). Mixed Type II cryoglobulinaemia is the major extrahepatic manifestation of HCV infection(Owlia 2007).it is a systemic vasculitis which manifests clinically by a classical triad of purpura, weakness and arthralgias(Ferri 2008). The term cryoglobulinemia refers to the presence of single or mixed immunoglobulins (Igs) in the serum, these (Igs) precipitate at a temperatures below 37°C and redissolve on rewarming. This is an in vitro phenomenon which can be observed in a wide spectrum of hematologic, infectious and immunorheumatologic disorders (Sansonno et al., 2005).

It has been established that low levels of circulating mixed cryoglobulins can be detected in over 50% of HCV-infected individuals (Sansonno et al., 2003) and were found to be 11 times more frequent in a group of HCV patients than their respective control population in a study by El-serag et al (2002). In the present study the results regarding the prevalence of cryoglobulinemia in HCV patients were 15% versus 4% in controls. This is in agreement with previous studies that recorded higher prevalence of circulating mixed cryoglobulins in HCV patients compared to their respective control population.

Detecting cryoglobulinemia in a percentage of 15% matches results of Gad et al., (2003) who reported that the prevalence of cryoglobulinemia in Egyptian HCV patients was 14%, and of Weiner et al., (1998) who detected cryoglobulins in 19% of their HCV patients. On the other hand a recent Egyptian study done by Meky et al., (2007) reported that prevalence of cryoglobulins in sera of chronic HCV patients was only 8.78%. The prevalence of HCV-related cryoglobulinemia varies greatly from one study to another with a prevalence of serum cryoglobulins ranging from 19% to >50% (Della Rossa et al.,
2001 ; Ferri et al., 2004 ; Tedeschi et al., 2007 and Fallahi et al., 2008), a finding that raises questions about the factors underlying its occurrence. This discrepancy in results may be due to different cryoglobulins detection methods from a study to another, but this cannot be the only reason for such different results. The involvement of particular HCV genotypes, environmental and/or host genetic factors should contribute to the pathogenesis of MC (Ferri, 2008). In this study the relatively hot climate in Egypt may play an important role in the results discrepancy between this study and the other European studies, in addition to prevalent HCV genotype 4 here in Egypt (90%), which is different from that prevalent in most Western countries, (Vinod, 2004 and El-Hawary et al., 2007). Another suggested cause is that patients of this study were not having HBV or HIV infections plus being relatively newly diagnosed cases.

Other environmental factors may play a role and genetic factors of different populations such as HLA typing may also be involved in the pathogenesis of cryoglobulinemia (Ferri et al., 2002; Liakana et al., 2002).

Mixed cryoglobulinemia can be detected in up to half of patients with HCV infection, but in 90% of cases it causes no symptoms and requires no specific treatment (Ali and Zein, 2005). Another study by Tedeschi et al., (2007) reported that 20-56% of patients with HCV had cryoglobulinemia and only 10-27% of them, showed clinical signs consistent with the syndrome of cryoglobulinemia.

The data in this study regarding clinical symptoms which are commonly accompanying cryoglobulinemic cases, generalized weakness and fatigue were present in 52% of all patients and in 8/15 (53.33%) of cryoglobulin +ve cases and so, it is considered the most prevalent symptoms and this was in general agreement with that of Mohammed et al.,(2010), Meky et al., (2007),and Stefanova-Petrova et al., (2007) as they reported that fatigue and weakness were the most prevalent symptoms related to Chronic Hepatitis C (CHC) patients.

In this study regarding incidence of clinical symptoms which are commonly accompanying cryoglobulinemic cases, generalized weakness and fatigue were present in 52% of all patients and in 8/15 (53.33%) of cryoglobulin +ve cases and so, it is considered the most prevalent symptoms and this was in general agreement with that of Mohammed et al.,(2010), Meky et al., (2007),and Stefanova-Petrova et al., (2007) as they reported that fatigue and weakness were the most prevalent symptoms related to Chronic Hepatitis C (CHC) patients.

Regarding extra hepatic manifestations (EHM) The findings in this study showed that 62% of the patients had one or more EHM, this was in general agreement with that of Cacoub et al., (2000) who reported that 40-74% of patients infected with HCV develop at least one EHM during the course of the disease, and also Stefanova-Petrova et al., (2007) reported that (76%) of the patients had at least one extra hepatic manifestation. the most common extra hepatic manifestations in this study were rheumatologic (19%) and cutaneous (17%).

Different studies report different frequencies of various dermatoses with chronic HCV infection patients. Azafar et al., (2008) noticed skin manifestations in 54% of their patients and generalized pruritus was the most common finding seen in 15.9% patients, Dervis and Serez, (2005) revealed pruritus to be most frequent symptom seen in 18.57% of patients. Other dermatoses included leukocytoclastic vasculitis and lichen planus seen in 4.28% each. In this study we detected other associated dermatological diseases in the patients as follows, Lichen Planus, Psoriasis, Urticaria, Necrolytic Acral Erythema (NAE) and Vitiligo with prevalence of (1%), (1%), (5%), (1%) and (2%) respectively. The most common dermatological symptom was pruritus in 36 (36%) of all patients and in 6 (40%) of cryopositive patients.
Circulating mixed cryoglobulins (MCs) were detected in 40-60% of HCV-infected patients whereas overt cryoglobulinaemic vasculitis developed in only 5-10% of the cases (Saadoun et al., 2007), the results of the present study showed that vasculitis incidence among all patients was 4%. In this study HCV RNA was detected in biopsies of the vasculitic skin lesions, by in situ hybridization in 3/4 (75%) of the cases, this finding could be explained by what was mentioned by Ferri et al (2003) about the hypothesis of a direct involvement of HCV in the immune-complex-mediated vasculitic lesions, as well as in the underlying lymphoproliferative disorder.

Conclusion:

We conclude that CHCV infection is an important risk factor for cryoglobulinaemia. Serum cryoglobulins presence should be searched for when mixed cryoglobulinemia is suspected in HCV patient complaining from generalized weakness, arthralgia, purpuric skin lesions, peripheral neuropathy, Reynaud’s phenomenon or renal troubles. The prevalence of cryoglobulinaemia in Egypt may be lower than other areas. The involvement of particular HCV genotypes (genotype 4 about 90%), environmental and/or host genetic factors should contribute to the pathogenesis of MC. Furthermore, a negative cryoglobulin test result does not exclude mixed cryoglobulinemia, as the test can be falsely negative.

References

علاق الكبد المتلازمة في الجلوبولينات البردية وتأثيرها على الجلد في المرضى المصريين

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قسم الأمراض اللولبية. قسم الأمراض الجلدية. المعهد القومي للأمراض المزمنة.

ال **)& عدد حالات الالتهاب الشديد الكبد في المرضى المصريين على عالم هو 170 مليون، مضافة إلى 300 مليون إصابة جديدة كل عام. في الوقت الحالي تعتبر عدد حالات الالتهاب الشديد الكبد في المرضى المصري على العالم أهم مشاكل الصحة العالمية وأعمال الأمراض الكبدية المتلازمة في جميع أنحاء العالم.

ويعتبر مصر أعلى دول العالم من حيث انتشار عدد حالات الالتهاب الكبد الفيروسي سي، حيث تصل نسبة الإصابة 8% من عموم الشعب المصري، من ديمو والهيئة العامة للأمراض المزمنة في جميع أنحاء العالم. وتختلف النسبة باختلاف الشريحة العمرية و باختلاف المسكن ما بين الريف و الحضر، وهذه النسبة في مصر تعادل تقريباً 0.1 أضعاف مثيلاتها في أي دولة أخرى من دول العالم.

وقد وجد أن الالتهاب الكبد الفيروسي سي له تبعات إكلينيكية متعددة ومتباينة جداً حوالى منه أكثر من مجرد مرض في الكبد، وقد تُحدث مع بعض الأعراض المختلفة خارج الكبد، وتكون هذه الأعراض ناتجة عن إصلاح أجزاء متعددة من الجسم مثل الكبد، الجلد، الدم، الجهاز الروماتيزمي. وطبقاً لعدد من الدراسات، وحده أن 40-74% من مرضى الالتهاب الكبد الفيروسي سي قد يعانون من عرض واحد أو أكثر من هذه الأعراض في فترة مرضية. وذلك من بين الأعراض التي تحدث خارج الكبد مع حالات الالتهاب الكبد الفيروسي سي، والتي تؤدي إلى حدوث التهابات بمختلف الأوعية الدموية بالجسم و التي قد تكون مصحبة بأعراض في صورة شعور بالإرهاق، طفح جلدي، آلام بال الصادر، ظاهرة رينو، التهاب الأوعية الدموية، أمراض الكلي أو أمراض الأعصاب العضدية.

والاطلق لدراسات عالمية عديدة، تبين أن وجود الجلوبولينات البردية في الدم هو أشهر الأعراض التي تحدث خارج الكبد مع حالات الالتهاب الكبد الفيروسي سي، ولكن بالنسبة لمدى شعوره بين مرضى الالتهاب الكبد الفيروسي سي محلياً في داخل مصر قد تم دراسة الموضوع بصورة واضحة المطلوبة.

الهدف من الدراسة:

تقييم وجود الجلوبولينات البردية ومدى شعورها في مصاب مرضى الالتهاب الكبد الفيروسي سي في مصر، وتأثيرها المحتمل على الجلد في صورة التهابات وعائية.

وقد شملت هذه الدراسة مجموعة مكونة من 16 مريض يعانون من الالتهاب الكبد الفيروسي سي في:

- أخذ تاريخ مرضي كامل.
- فحص جدي شامل (بحث عن التهابات وعائية).
- أخذ عينات من الجلد المصاب بالالتهابات الوعائية.
- تحقي جهاز دم.
- عمل وظائف حمية.
- عمل فحص بالوجوات فوق الصوتية على البطن.
- البحث عن الأجسام المضادة للالتهاب الكبد الفيروسي في عينات الدم.
- عمل PCR لتحديد كمية الفيروس في أمصال المرضى.
- عمل اختبار الترسيب للجلوبولينات البردية في أمصال المرضى.

- 206 -
عمل PCR للعينات المأخوذة من الجلد المصاب.
ثم التحليل الإحصائي للنتائج.

وقد أظهرت هذه الدراسة وجود الجلوبيولينات البردية في 15% من مرضى الالتهاب الكبدي المزمن الفيروسي سي و هي نسبة عالية إذا ما قورنت بالمجموعة الضابطة والتي وجدت فقط في 4% من أفرادها، و لقد وجد الحمض النووي الخاص بالفيروس في 3 من أصل 4 عينات جلدية (التهاب وعائي) من المرضى الإيجابيين للجلوبيولينات البردية.

و لقد أوصت هذه الدراسة بالأتي:

• عمل اختبارات معملية للبحث عن الجلوبيولينات البردية في مرضى الالتهاب الكبدي الفيروسي سي.
• عمل اختبارات معملية للبحث عن فيروس سي في المرضى الإيجابيين للجلوبيولينات البردية.
• عمل المزيد من الدراسات لتوسيع أثر هذه الجلوبيولينات البردية على كيفية سريان أو تطور مرض الالتهاب الكبدي الفيروسي سي.