Evaluating anti β-2 glycoprotein as rapid thromboembolic marker among HCV patients in Egypt

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To cite this article:

Abstract: Although antiphospholipid (APL) antibodies are still not clearly known, yet they have association with thromboembolic events associated with hepatitis C virus (HCV) infection. Moreover, high percent of HCV infections and thromboembolic risk was associated among thalassemic patients. So our aim was to screen and evaluate the significance of ACL and anti β-2 glycoprotein seroprevalence among patients receiving repeated blood transfusions, in patients with infectious diseases as chronic HCV disorders, and in healthy subjects resident in Egypt, a tropical country endemic for hepatitis and to evaluate these tests as rapid markers for early detection of thrombotic HCV infection among chronic liver disorders and cases receiving repeated blood transfusion aiming at early anticoagulant therapy. The study was carried out on 70 patients attending Tropical, Hepatology and Hematology departments in Alexandria Main University Hospital, Egypt as well as 30 healthy subjects served as controls. Group I; 35 chronic liver disorders as chronic HCV, cirrhosis and Hepatocellular carcinoma(HCC) diagnosed by clinical, ultrasound and histopathology. Group II ; 35 receiving repeated blood transfusion as thalassemia. Group III; healthy relatives –ve for HCV antibody of matched age and sex. Both markers were screened by commercial enzyme immunosorbant assays (ELISA) (Calbiotech,USA). Our results revealed that patients with chronic HCV infection had higher anticardiolipin (ACL) IgG and antibeta2GP1 IgG compared to healthy controls. (26.6%) 8 patients from group I had high ACL IgG compared to 6 patients (20%) of group II .The study also revealed that anti β-2 glycoprotein IgG was higher among Group I and II compared to controls group III. Group I was higher than group II. Three patients (10%) of group I had high antibeta2 glycoprotein1 IgG compared to 2 patients (6.6%) among group II . We can conclude that anticardiolipin (ACL) alone is not diagnostic for thrombosis & it should be accompanied with antibeta(2 glycoprotein-1 in serum of the same patient to be considered a cause of thrombosis in chronic HCV patients. So, ACL alone can not be considered rapid marker of thrombosis in HCV positive patients. We recommend anti β2 glycoprotein as a biomarker predicting thrombotic HCV infection.

Keywords: Anti β2glycoprotein , ACL, ELISA, HCV, RT-PCR, Thromboembolic Rapid Markers

1. Introduction

Antiphospholipids antibodies (APL) are circulating immunoglobulins that bind to negatively charged phospholipid and promote abnormal coagulation that predisposes to arterial or venous thrombosis. Anticardiolipin (ACL) is the most commonly investigated APL in relation to stroke and transient ischemic attacks. Although several well conducted studies have demonstrated the association of ACL with a prothrombotic state and stroke, (1) the pathogenesis and the risk prediction in ischemic stroke are still debatable(2,3).

Several reports have clearly shown that several viral, bacterial, and parasitic infections are associated with generation of APL(4-8).

A high prevalence of APL was observed in inhabitants of regions such as tropical Africa, where there is high endemicity of infectious diseases(4-6). Although these infections can induce APL expression, the increase in antibodies was not accompanied by manifestations of the antiphospholipid syndrome (APS) such as thrombosis(9).

People with APS make antibody to beta-2 glycoprotein I. Beta-2 glycoprotein I binds to phospholipids that “ flipped “ to outside of cell.
Elevated levels of ACL were found by several investigators in HCV patients and these were all beta-2glycoprotein I (β2GPI)-independent(10).

Although the precise nature of APL is still not known, yet reported association with thromboembolic events and hepatitis C virus (HCV) infection was issued by some researchers. Moreover, high HCV infection and thrombotic risk was described among thalassemic patients(11,12).

Hepatitis C virus (HCV) was recognized as a major threat to the whole globe. It has been estimated that 170 million people were infected worldwide, most of them chronically infected and at risk for liver cirrhosis and hepatocellular carcinoma (HCC).

The highest prevalence was among Egyptians; 10 - 20% of the population were chronically infected, some with HCC and others were cirrhotic (13,14).

Our aim was to screen and evaluate the significance of ACL and anti β-2 glycoprotein among patients receiving repeated blood transfusions like thalassemia, in patients with infectious diseases as chronic HCV disorders, and in healthy subjects resident in Egypt, a tropical country endemic for hepatitis.

Evaluating these tests as rapid markers for early detection of thrombotic HCV infection among chronic liver disorders and cases receiving repeated blood transfusion will aid clinicians at early anticoagulant therapy preventing thromboembolic manifestations.

2. Subjects and Methods

The current research was carried out on 70 patients attending Hematology, Hepatology and Tropical Departments, Alexandria Main University Hospital, Egypt as well as 30 healthy subjects served as controls.

An informed consent from all enrollees was taken and approved by our ethical committee.

Case selection was done according to the following criteria: Male or female aged from 20-60 years, HCV antibody and PCR (polymerase chain reaction) positive.

Group I ; 35 receiving repeated blood transfusion (thalassemia major),

Group II ; 35 chronic liver disorders as chronic HCV, cirrhosis and HCC diagnosed by histopathology and Group III; healthy relatives –ve for HCV antibody of matched age and sex. All enrollees were subjected to: history taking (Personal- medical- surgical and therapeutic), blood samples were drawn for laboratory investigations(6,8);

Estimating both ACL (IgM and IgG) and anti beta 2 glycoprotein (Antiβ2GPI) in sera by commercial enzyme linked immunosorbent assay (ELISA), Calbiotech, USA.

Principle of ELISA; It was an indirect solid phase enzyme linked immunosorbant assay for the quantitative measurement of IgG and IgM against cardiolipin and beta2 glycoprotein (Antiβ2GPI) in human sera. We followed the manufacturer instructions which was as follows:

Highly purified antigen as cardiolipin or β2glycoprotein were bound to microwells. Several washing of microwells removed unbound serum antibodies. Then we added anti-human IgG and IgM conjugated to horseradish peroxidase which bound to patient antibodies forming a conjugate/antibody/antigen complex. Washing microwells several times removed the unbound conjugate. Finally we added an enzyme substrate which in the presence of bound conjugate was hydrolyzed to form a blue color. The addition of an acid stopped the reaction forming a yellow end-product. The intensity of this yellow color was measured photometrically by an ELISA reader at 450 nm (Behring, Germany). The amount of colour was directly proportional to the concentration of IgG and IgM antibodies present in the original sample.

Statistical tests: was performed using SSPS version 15 Microsoft, USA.

3. Results

The current study revealed the following data:

Table (1) illustrates ACL and Antiβ2GPI % among the three studied groups, ACL ranged between 16.30-24.50, 12.10-20.0 and 4.30-11.70 with the mean of 20.01±2.57, 16.61±2.53 and 8.65±2.24 for the three studied groups. The first 2 groups (cases) revealed statistically higher values than group III (controls), also group I values were higher than group II. (P=0.0001).

Table 1. Comparing both markers (ACL and Antiβ2GPI) among studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticardiolipin antibodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I (no.35)</td>
<td>20.0133</td>
<td>2.57665</td>
<td>16.30</td>
<td>24.50</td>
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<tr>
<td>Group II (no.35)</td>
<td>16.6133</td>
<td>2.53578</td>
<td>12.10</td>
<td>20.00</td>
</tr>
<tr>
<td>Control (no.30)</td>
<td>8.6500</td>
<td>2.24818</td>
<td>4.30</td>
<td>11.70</td>
</tr>
<tr>
<td>F</td>
<td>168.923</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>.0001*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5.6200</td>
<td>1.76545</td>
<td>2.50</td>
<td>9.00</td>
</tr>
<tr>
<td>Antiβ2 glycoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>3.5933</td>
<td>1.78614</td>
<td>1.20</td>
<td>7.00</td>
</tr>
<tr>
<td>Control group</td>
<td>2.3867</td>
<td>.95076</td>
<td>.60</td>
<td>4.00</td>
</tr>
<tr>
<td>F</td>
<td>33.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>.0001*</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*P is significant ≤ 0.05.

While Antiβ2GPI % among the three studied groups ranged between 2.50-9.0, 1.20-7.0 and 0.60-4.0 with the mean of 5.62±1.7, 3.59±1.78 and 2.38±0.95 for the 3 studied groups. Cases: groups I & II had values statistically higher than group III (control group), meanwhile group I values were higher than group II. (P=0.0001).

Correlations between different studied parameters were
presented in table (2) showed correlations between different studied parameters, it shows that, there was positive significant correlation between anti β-2 GP and ACL antibodies (P=0.0001), while there was no significant correlation between HCV load done quantitatively by real time polymerase chain reaction (RT-PCR) and both ACL antibodies and anti β-2 GP. (P=0.305 and 0.649 respectively).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti β-2 Glycoprotein</td>
<td>.531(*)</td>
<td>.0001</td>
</tr>
<tr>
<td>HCV by RT-PCR</td>
<td>.284 -.128</td>
<td>.305 .649</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

Hepatitis C is a viral infection transmitted parentally by the hepatitis C virus (HCV), which has six major genotypes that infect human cells, including hepatocytes, B lymphocytes, dendritic cells, monocytes, and epithelial cells (15,16). Millions of people in the world are infected with this virus (17).

Infection with HCV include: i) persistence of the virus, which may cause cirrhosis and hepatocellular carcinoma and ii) autoimmunity, which is mainly represented by cryoglobulinemia and production of non-organ-specific autoantibodies, including APL autoantibodies (18-20).

APL antibodies are a group of immunoglobulins that binds to epitopes of proteins complexed with anionic phospholipids or epitopes of anionic phospholipids. Pathogenic antiphospholipid antibodies are immunoglobulins that react with proteins of the coagulation system such as β2-glycoprotein I (β2GPI) or prothrombin, causing a coagulation disorder known as antiphospholipid syndrome.

HCV persistence evokes several autoantibodies and concomitant autoimmune disorders. Autoantibodies such as non-organ-specific and liver-specific have been demonstrated in sera of patients with HCV-related chronic liver disease (CLD).

Possible mechanisms for these autoantibodies are Molecular mimicry between self proteins and HCV polyprotein, and polyclonal B cell activation by chronic HCV infection.

Some of these autoantibodies are tightly associated with concurrent autoimmune diseases, and others are closely associated with human leucocyte antigen (HLA).

Our results revealed that chronic HCV patients had significantly higher serum levels of ACL IgG and antibeta2GP1 IgG compared to healthy controls.
Comparison between the three studied groups for ACL antibodies demonstrated that Groups I and II revealed values statistically higher than controls, also group I showed values statistically higher than group II.

The study revealed that (26.6%) 8 patients from group I have high serum anticardiolipin IgG while 6 patients (20%) of group II have high anticardiolipin IgG. Also 3 patients (10%) of group II have high serum anticardiolipin IgG while 2 patients (6.6%) have high serum antibeta2glycoprotein I IgG.

The increased ACL levels among HCV patients in the present study were similar to other investigators done elsewhere(21,22). The elevated ACL levels may cause recurrent venous or arterial thrombosis by binding to negatively charged phospholipids of the cell membrane. Its clinical significance was not settled. While some studies, including the present one, suggest the absence of thrombosis among viral infections(23,24).

The presence of ACL among viral or other infections might be induced by disturbances in the regulation of cellular and humoral immunity, which were the consequence of infectious diseases(15). In patients with chronic viral hepatitis, induction of neoantigens may provoke antibody formation by disrupting liver cell membrane(25). Moreover, viruses stimulate apoptosis this may lead to redistribution of plasma membrane phospholipids and their over expression on the apoptotic cell membrane surfaces resulting in ACL formation(22).

The present study also showed that serum levels of antibeta2GPI antibody increased among viral hepatitis patients. Such a finding was in agreement with previous reports examining the levels of the antibody in various viral infections. Viral infections were believed to induce a lower antibeta2GPI than APS or other autoimmune diseases (26,27).

Beta2GPI is a single chain glycoprotein with amino acid residues arranged in five homologous domains called Sushi domains. It was suggested that ACL and antibeta2GPI antibodies recognize neo epitopes on Sushi-4 domain induced after binding of antibeta2GPI antibody to the lipid surface(28-30).

The current study also illustrated that anti β-2 glycoprotein ranged between 2.50-9.0, 1.20-7.0 and 0.60-4.0 with the mean of 5.62±1.7, 3.59±1.78 and 2.38±0.95 for the three studied groups. Cases revealed higher values than controls, also group I have values statistically higher than group II. (P=0.0001).

Correlations between different studied parameters were presented in table (3) it showed that, there was significant positive correlation between anti β-2 glycoprotein and ACL antibodies (P=0.0001), while there were no statistical significant correlation between HCV positive by RT-PCR and each of ACL antibodies and anti B-2 glycoprotein. (P=0.305 and 0.649 respectively).

Comparison between the negative and the positive HCV cases(by PCR) regarding ACL and anti B-2 glycoprotein antibodies was done to show if HCV viraemic level of the patient could affect their levels. It showed that Anticardiolipin antibodies ranged between 16.3-24.5 and 16.3-24.4 with the mean of 19.95±2.60 and 20.07±2.64 for positive and negative HCV by PCR respectively. While there was statistical significant difference among anti β-2 glycoprotein compared to controls. Our results were similar to those done elsewhere(31-32).

No one of our studied patients had history of arterial or venous thrombosis. Also no one had high level of both ACL and Antibeta2GP1 together.

To conclude, some patients with viral hepatitis infections may produce ACL and or antibeta2 glycoprotein as an epiphenomenon of the infectious diseases. Such patients may not show clinical signs of venous or arterial thrombosis.

Thus we can conclude that antibeta2GP1 antibody may be considered a determinant factor in the production of thromboembolic manifestation in hepatitis C associated ACL and we recommend it as rapid biomarker to guide clinicians for early anticoagulant to prevent thromboembolic manifestations.

References


4  Elkhouly EH et al.: Evaluating anti β-2 glycoprotein as rapid thromboembolic marker among HCV patients in Egypt


