Autoimmunity in hepatitis C virus carriers: Involvement of ferritin and prolactin

Gabriel M. Sousa, Rodrigo C. Oliveira, Mariana M. Pereira, Raymundo Paraná, Maria Luiza B. Sousa-Atta, Ajax M. Atta

Abstract

Background: Ferritin and prolactin have been associated with active autoimmune diseases as systemic lupus erythematosus and autoantibody production, but have been little studied in viral infections that present autoimmunity.

Objective: To investigate the association of these two autoimmune mediators with the presence of cryoglobulinaemia and non-organ-specific autoantibodies (RF, SMA, β2GPI IgA antibody and ANA) in Brazilian individuals chronically infected with hepatitis C virus (HCV).

Methods: Ninety-nine patients were evaluated. Ferritin and prolactin levels were determined by chemiluminescent immunoassays.

Results: Hyperprolactinemia was found in 10 (six men and four women) out of 99 (10.1%) hepatitis C patients. Thirty-eight out of 99 (38.4%) HCV carriers had hyperferritinemia (median level 385 ng/mL). Neither hyperprolactinemia nor hyperferritinemia was associated with cryoglobulinaemia or non-organ-specific autoantibodies (p > .05). There was an association between hyperprolactinemia and the infection with HCV genotype 3 (p < .01). Ferritin and ALT levels were correlated (p < .05).

Conclusion: Our results suggest that neither prolactin nor ferritin is involved with the extra-hepatic manifestation of autoimmunity observed in HCV carriers.

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1. Introduction

The chronic infection caused by hepatitis C virus affects around 170 million people worldwide according to the World Health Organization. The persistence of HCV in untreated patients can cause B cell
disfunction characterized by lymphoproliferation and extra-hepatic manifestations of autoimmunity that are mainly represented by mixed cryoglobulinemia and production of non-organ-specific autoantibodies as smooth muscle antibodies, antinuclear and/or antiphospholipid antibodies. Among the common clinical manifestations presented by cryoglobulinemic HCV patients are systemic vasculitis, membranous proliferative glomerulonephritis (MPGN) and peripheral neuropathy. Furthermore, there is an association of chronic HCV infection with celiac disease, arthritis and Sjögren’s syndrome [1–8].

Prolactin and ferritin are two important bioactive molecules whose association with autoimmune diseases and production of autoantibodies has been studied. Prolactin induces the production of IL-1 and interferon-γ, promotes the expression of IL-2 receptor, participates in the pathogenesis of autoimmune diseases stimulating anti-apoptotic activity and impairment of the negative selection of autoreactive B cells, and stimulates autoantibody production. In systemic lupus erythematous, prolactin seems to modulate IFN-γ secretion and to change the phenotype of dendritic cell from an antigen presenting cell to a proinflammatory cell [9–12]. Ferritin is an iron storage protein involved in acute inflammation that has been reported as a biomarker of SLE activity. Nevertheless, ferritin can down-modulate both T and B cell immune response, suppressing type IV hypersensitivity and autoantibody production, respectively, and reduces granulocyte phagocytosis and stimulates IL-10 production by CD4+ CD25+ regulatory T cells [13–16].

The association of prolactin and ferritin with autoimmunity in diseases caused by viral pathogens as HCV is little studied. To verify their involvement in the expression of autoimmunity in chronic hepatitis C, we investigated the existence of association between hyperprolactinemia and hyperferritinemia and cryoglobulinemia and NOSA production in patients chronically infected with HCV.

2. Materials and methods

2.1. Patients

Ninety-nine patients, 56 men (median age 47 yr, IQR = 44–51 yr) and 43 women (median age 49 yr, IQR = 40–58 yr) were the target group. They had clinical diagnosis and laboratory findings of chronic hepatitis C represented by a positive anti-HCV test of third generation enzyme-linked immunosorbent assay (ELISA) (AXSYM System; Abbott Laboratories, Chicago, IL, USA) and a positive RNA-Polymerase Chain Reaction (Amplicor® HCV Detection Kit V2.0; Roche Molecular Systems Inc., Somerville, NJ, USA). All patients signed an informed consent to participate of this study that was approved by an Ethnic local Committee.

2.2. HCV genotyping

Hepatitis C virus genotypes were identified using the Inno-LiPA test (HCV LineProbe Assay; Innogenetics, Zwijndrecht, Belgium).

2.3. Biochemical and immunological assays

Serum levels of prolactin and ferritin were determined using automated chemiluminescent immunoassays (Access 2; Beckman–Coulter, USA). The reference values for prolactin were 2.0–15.0 ng/mL for men and 2.0–25.0 ng/mL for women, whereas the reference values for ferritin were 36–262 ng/mL for man and 34–155 ng/mL for woman. Serum alanine aminotransferase (ALT) level was determined by biochemical automated analysis. The presence of cryoglobulinemia was tested by tube cryoprecipitation during 7 days at 4 °C. Smooth muscle antibodies were probed by indirect fluorescent antibody, while ANA and IgA anti-β2 glycoprotein I were probed by commercial kits of indirect ELISA (INOVA Diagnostics Inc., San Diego, CA, USA). Rheumatoid factor was tested by nephelometry (Image Nephelometer; Beckman–Coulter, USA).

2.4. Statistical analysis

Statistical analysis was performed with the GraphPad software 5.03. Results were expressed as median and interquartile range (Q1–Q3). Categorical groups were analyzed for association and correlation with Fisher’s exact test and the Spearman test, respectively. Significant level was set at p < 0.05.

3. Results

The medians of the serum level of alanine aminotransferase (ALT) for men and women were 42.5 U/L (IQR = 26–74.2 U/L) and 27 U/L (IQR = 16–50 U/L), respectively. The following prevalence of HCV genotypes was observed: HCV genotype 1 (67%), genotype 3 (29%) and genotype 2 (4%). Cryoglobulinæma was detected in 63 out of 99 (63.3%) patients. The following seropositivity for non-organ-specific autoantibodies was observed: rheumatoid factor (54.99, 54.5%), smooth muscle antibody (29.99, 29.3%), IgA anti-β2GPI (26.99, 26.3%) and ANA (18.99, 18.1%). The serum level of prolactin presented a median of 6.4 ng/mL (IQR = 4.8–9.2 ng/mL) in the man group and of 7.4 ng/mL (IQR = 4.8–10.1 ng/mL) in the woman group. Hyperprolactinæma was detected in 10 patients, six men and four women (Fig. 1). The serum level of ferritin was 260 ng/mL (IQR = 135–400 ng/mL) in the men and of 115 ng/mL (IQR = 39–224 ng/mL) in the women, and hyperferritinæma was demonstrated in 38 out of 99 (38.4%) patients (median = 385.3 ng/mL, IQR = 299–651 ng/mL) (Fig. 2). No association was observed between hyperprolactinæma or hyperferritinæma and the autoimmune findings (Tables 1 and 2, p > 0.05). There was an association between hyperprolactinæma and the infection with HCV genotype 3 (p < 0.01). A direct correlation was observed between ALT and ferritin level (r = 0.216, p < 0.05).

4. Discussion

Autoimmunity is a common finding in chronic hepatitis C. Such a B cell dysfunction may be caused by an interaction of B cells with HCV, which modulates the function of this cell, promotes its polyclonal activation and expands CD5+ cells [4,17]. However, the immunopathogenesis of this extra-hepatic clinical manifestation of chronic HCV infection still deserves more studies. In this work, we investigated the association of hyperprolactinæma and hyperferritinæma with the autoimmune findings presented by men and women chronically infected with HCV.

Despite the reports of the involvement of prolactin in the immune response and autoimmunity [9–12], we did not find an association of increase serum prolactin levels with cryoglobulinæma or NOSA production (RF, SMA, β2GPI IgA antibody and ANA), findings that
have been associated with increased levels of BAFF, a cytokine that supports the proliferation of autoreactive B cells [18–23]. The absence of hyperprolactinemia in cryoglobulinaemic patients contrasts with the reports showing its important prevalence in patients with active systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis or Sjögren syndrome [24], and suggests the non-involvement of the hypothalamus–adrenal axis in these extra-hepatic manifestations of autoimmunity in chronic hepatitis C. Interestingly, the lack of association of NOSA with hyperprolactinemia reinforces the idea that NOSA production is an epiphenomenon caused by molecular mimicry between HCV polyprotein and some target self nuclear antigens (matrin722-741, histone H2A11-30, replication protein A133-152) and some smooth muscle antigens (smoothelin698-717, myosin1035-1054, vimentin69-88) [25].

The association of hyperprolactinemia with HCV genotype 3 was unexpected because there is no evidence that HCV genotypes differ in either immunopathogenesis or clinical presentation of their infections. However, this laboratory finding needs to be better investigated to validate its importance in the pathogenesis of chronic hepatitis C.

The presence of hyperferritinemia in around one-third of the patients with chronic hepatitis C is in agreement with a previous report, which also demonstrated a direct correlation between ferritin and ALT levels in this population [26]. This finding and the demonstration that hyperferritinemia was not associated with a B cell dysfunction represented by cryoglobulinaemia and NOSA production, suggests that in chronic hepatitis C this protein may facilitate HCV persistence through its suppressive effects on the immune response of both T and B cells against this virus [14–16].

5. Conclusion

Both hyperprolactinemia and hyperferritinemia are not associated with the B cell dysfunction represented by cryoglobulin and non-organ-specific-antibody production observed in patients chronically infected with HCV.

Table 1

<table>
<thead>
<tr>
<th>Finding</th>
<th>Normal prolactin</th>
<th>High prolactin</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryoglobulin</td>
<td>56/63</td>
<td>7/63</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>RF</td>
<td>47/54</td>
<td>7/54</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>ASMA</td>
<td>26/29</td>
<td>3/29</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>IgG2 Gp IgA</td>
<td>25/26</td>
<td>1/26</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>ANA</td>
<td>18/18</td>
<td>0/18</td>
<td>&gt;.05</td>
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</tbody>
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Association between two categorical groups was analyzed by the Fisher’s exact test.

6. Authors’ contributions

AMA takes responsibility for the study integrity and data analysis accuracy. GMS, RCO performed laboratory analysis. RP performed the clinical evaluation of the patients and MLSA participated in study supervision. All authors read and approved the final manuscript.

Take-Home messages

- Cryoglobulinaemia and NOSA are common in patients chronically infected with hepatitis C virus.
- Hyperprolactinemia and hyperferritinemia have been associated with autoimmune diseases and autoantibody production.
- Neither hyperprolactinemia nor hyperferritinemia is associated with the extra-hepatic manifestation of autoimmunity in HCV carriers.

Acknowledgments

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References


Table 2

<table>
<thead>
<tr>
<th>Finding</th>
<th>Normal ferritin</th>
<th>High ferritin</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryoglobulin</td>
<td>41/63</td>
<td>22/63</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>RF</td>
<td>33/54</td>
<td>21/54</td>
<td>&gt;.05</td>
</tr>
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<td>ASMA</td>
<td>20/29</td>
<td>9/29</td>
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Association between two categorical groups was analyzed by the Fisher’s exact test.
Follistatin-like protein 1 is elevated in systemic autoimmune diseases and correlated with disease activity in patients with rheumatoid arthritis

Follistatin-like protein 1 (FSTL1) is a proinflammation mediator implicated in arthritis in rodent animal models. In this regard, Li D, et al. (Arthritis Res Ther 2011;13: R17) aimed at assessing FSTL1 levels in systemic autoimmune diseases and correlating them with disease activity in patients with rheumatoid arthritis (RA). Serum FSTL1 levels from 487 patients with systemic autoimmune diseases and 69 healthy individuals were measured by enzyme-linked immunosorbent assay (ELISA). FSTL1 expression was shown to be higher in systemic lupus erythematosus (SLE), Sjögren syndrome (SS), systemic sclerosis (SSc) and polymyositis/dermatomyositis (PM/DM). Serum FSTL1 levels in RA patients were significantly higher than those in other systemic diseases, suggesting that serum FSTL1 levels may serve as biomarkers of disease activity.

Reorganization in cognitive networks with progression of multiple sclerosis: Insights from fMRI

Cognitive dysfunction (CD) is frequent in multiple sclerosis (MS) and can occur at early stages. Whereas functional reorganization with disease progression has been described for the motor system in MS using fMRI, no such studies exist for cognition. Loitfelder M, et al. (Neurology 2011;76:526-33) attempted to assess the concept of functional reorganization concerning cognition using a simple “Go/No-go” fMRI paradigm. Patients with a clinically isolated syndrome (CIS, n = 10), relapsing-remitting MS (RRMS) (n = 10), or secondary progressive MS (SPMS) (n = 10), and 28 healthy controls (HC), underwent a comprehensive neuropsychological test battery, clinical examination, structural imaging, and an fMRI Go/No-go discrimination task at 3 T. Patients performed worse than HC regarding memory, sustained attention, and concentration, and information processing. These differences were driven by patients with SPMS. The fMRI task elicited activation in a widespread network including bilateral mesial and dorsolateral frontal, parietal, insular, basal ganglia, and cerebellar regions. Task performance was similar between phenotypes, but deviation from the activation pattern observed in HC and patients with CIS increased with disease progression. Patients with RRMS showed increased brain activation in the precuneus, both superior parietal lobes, and the right fusiform gyrus, and recruited the hippocampus with increasing demands. Patients with SPMS demonstrated the most abnormal network function, including recruitment of pre-SMA, bilateral superior and inferior parietal, dorsolateral prefrontal, right precentral, bilateral postcentral, and right temporal brain areas. Using a cognitive fMRI paradigm, authors were able to confirm adaptive changes of neuronal activation with progressing MS and to provide strong evidence for their compensatory nature, at least partially.

Moving towards a cure: blocking pathogenic antibodies in systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is characterized by the presence of autoantibodies that can mediate tissue damage in multiple organs. The underlying etiology of SLE remains unknown, and treatments aimed at eliminating B cells, or limiting their function, have demonstrated limited therapeutic benefit. Thus, the current therapies for SLE are based on the concept of nonspecific immunosuppression and consist of nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, anti-malarials and cytotoxic drugs, all of which have serious adverse side effects including organ damage. The major auto-specificity in SLE is double-stranded (ds) DNA. Many anti-dsDNA antibodies react with non-DNA antigens that may be the direct targets for their pathogenic activity. Studying anti-dsDNA antibodies present in SLE patients and in animal models of lupus. Diamond B, et al. (J Intern Med 2011; 269: 36-44) have identified a subset of anti-dsDNA antibodies which is pathogenic in the brain as well as in the kidney. They have recently demonstrated that specific peptides, or small molecules, can protect target organs from antibody-mediated damage. Thus, it might be possible to treat aspects of autoimmune disease without inducing major immunosuppression and ensuing infectious complications.