CLINICAL STUDY

The presence of thyroid peroxidase antibody of IgG2 subclass is a risk factor for thyroid dysfunction in chronic hepatitis C patients

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Abstract
Objective: To investigate the prevalence of thyroid dysfunction (TD) and IgG subclasses of thyroid autoantibodies (TAs) and to determine the predictive factors of TD in chronic hepatitis C (CHC) patients.

Design: Three hundred and twelve untreated hepatitis C virus-infected patients without a history of TD or treatment with thyroid hormones were enrolled in a cross-sectional study. Clinical and biological factors were statistically analyzed to determine the correlation between TD and this patient population.

Results: The incidence of TD was 12.5% in CHC patients. Clinical hypothyroidism (5.8%) and subclinical hypothyroidism (3.8%) were more frequent than clinical hyperthyroidism (1.6%) and subclinical hyperthyroidism (1.3%). The percentage of TA-positive patients was significantly higher in people 60 years than in those ≤60 years (31.9 vs 18.6%; P=0.042). Positive thyroid peroxidase antibody (TPOAb) was more frequent, and alanine aminotransferase (ALT) levels were lower in patients who displayed TD (TPOAb: 62.1 vs 10.8%, P=0.000; ALT: 43.5 vs 51 IU/l, P=0.046). The positive percentage of TPOAb IgG2 subclass in the TD group was significantly higher than that of patients without TD (66.7 vs 16.7%, P=0.005). Multiple logistic regression analysis indicated that only TPOAb IgG2 subclass positivity was an independent risk factor for TD in CHC patients (odds ratio = 8; 95% CI: 1.225–52.246; P=0.030).

Conclusions: TPOAb IgG2 subclass positivity is a risk factor for TD in CHC patients before antiviral treatment. IgG2 subclass of TPOAb might play an important role in the presence of TD in CHC patients.

Introduction

Chronic hepatitis C (CHC) is one of the common infectious liver diseases and is a major global health problem (1). Hepatitis C virus (HCV) is recognized as the causative agent of CHC since its discovery in 1989 (2). The prevalence of HCV infection is 2.2% worldwide, and more than one million new cases have been reported annually by the WHO. About 25% of hepatocellular carcinoma (HCC) and 27% of cirrhosis worldwide occur in HCV-infected patients (3).

HCV infection frequently causes multiple extrahepatic manifestations such as autoimmune thyroiditis, insulin resistance, mixed cryoglobulinemia (MC), thrombopenia, aplastic anemia, and peripheral neuropathy (4, 5). Thyroid dysfunction (TD) and the emergence of thyroid autoantibodies (TAs), such as thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TGAb), are very common in CHC patients (6, 7, 8). Recent studies have focused on whether interferon (IFN) treatment with or without ribavirin (RBV) can induce thyroiditis in CHC patients. In fact, many patients with CHC experience thyroid problems even in the absence of IFN treatment (9, 10, 11). Ganne-Carrie et al. (12) reported that the incidence of TD and/or TAs was significantly higher in patients with hepatitis C infection who had not received IFN-α therapy. Furthermore, Nagane et al. (13) indicated that thyroid autoimmunity was linked to HCV infection and not to antiviral alone because antithyroid antibodies appeared in both treated and untreated CHC patients. The pathogenesis of TD in CHC patients triggered by HCV remains unclear. It has been reported that HCV can affect thyroid function by modulating T-cell and B-cell antibody responses, thus affecting TA profiles (10, 14). Indeed, IgG subclasses of TAs can have different biological functions.
The distribution of IgG subclasses of TPOAb/TGAb might provide insight into the mechanisms of TD in patients with HCV infection (15).

In this cross-sectional study, the frequency of TD and IgG subclasses of TAs in CHC patients before IFN (alone or plus RBV) treatment was assessed. The risk factors of developing TD in this population were further analyzed. These results may provide insights into the possible mechanisms of TD in CHC patients.

**Materials and methods**

**Patients**

A total of 312 CHC patients admitted to the Department of Infectious Diseases, Peking University First Hospital during July 2005 to August 2010 were enrolled in the current cross-sectional study. These patients came from four different regions in northern China (Beijing, Hebei province, Gansu province, and Shanxi province). The criteria of CHC diagnosis were according to the Guideline of Prevention and Treatment of Hepatitis C (16). All the patients had HCV antibodies and/or HCV RNA positive for more than 6 months, and none of them had a history of thyroid gland dysfunction. They did not receive thyroid hormones or antiviral therapy with IFN (alone or in combination with RBV) during the past years. Exclusion criteria included coinfection with hepatitis B virus and/or human immunodeficiency virus, other causes of chronic liver disease, other autoimmune diseases, and coexistence of serious psychiatric or medical illnesses. The study was in compliance with the Helsinki Declaration and was approved by the Medical Ethics Committee of Peking University First Hospital. All enrolled patients gave written informed consent.

**Laboratory assessment**

Venous blood was drawn to determine serum levels by an automatic biochemical analyzer of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin (TBIL and DBIL), total protein (TP) and albumin (ALB) after an overnight fast of 12 h (4).

HCV antibodies were analyzed by microsomal chemiluminescence (Abbott Diagnostics Division), and HCV genotyping was performed by restriction fragment length polymorphism analysis (5). Serum HCV RNA was measured by a real-time PCR assay (COBAS Taqman HCV Test; Roche Molecular Systems, Inc.).

Chemiluminescent immunoassays were used to detect TSH, free triiodothyronine (FT$_3$), free thyroxine (FT$_4$), and TPOAb/TGAb (TSH, FT$_3$, and FT$_4$ by ADVIA Centaur (Bayer Healthcare Diagnostics), TPOAb/TGAb by IMMULITE 1000 (Diagnostic Products Corporation, Los Angeles, CA, USA)). Clinical hypothyroidism was defined as TSH higher than 5.5 μIU/ml and FT$_4$ lower than 11.48 pmol/l, while clinical hyperthyroidism was diagnosed when TSH was lower than 0.35 μIU/ml and FT$_4$ was higher than 22.7 pmol/l and/or FT$_3$ was higher than 6.5 pmol/l. Subclinical hypothyroidism or hyperthyroidism was considered when the serum concentrations of TSH were higher than 5.5 μIU/ml or lower than 0.35 μIU/ml respectively with normal levels of FT$_3$ and FT$_4$. TAs were considered positive when TPOAb was higher than 35 IU/ml or TGAb was higher than 40 IU/ml.

**Assays of IgG subclasses of TPOAb/TGAb**

ELISA was used to detect specific IgG subclasses of TPOAb/TGAb: plates (Costar, Cambridge, MA, USA) were coated with 0.5 mg/ml TPO/TG (AppliChem Corporation, Ottoweg, Darmstadt, Germany) in 0.1 M carbonate/bicarbonate buffer (pH 9.6) at 4°C overnight. Serum samples were diluted (1:50) in PBS containing 0.1% Tween 20 (PBST) and incubated at 37°C for 30 min. After washing three times in PBST, HRP-labeled mouse anti-human MABs were added. MABs to IgG1 (4E3), IgG2 (HP6014), IgG3 (HP6050), and IgG4 (HP6025) (SouthernBiotech, Birmingham, AL, USA) were used at dilutions of 1:2000, 1:800, 1:800, and 1:2000, 1:1000, and 1:1000 in TPOAb series and 1:2000, 1:800, 1:800, and 1:5000 in TGAb series respectively. After incubation (37°C) for 30 min and extensive washing, 0.4 mg/ml o-phenylenediamine were added and the reaction was stopped with 1 M HCl after 20 min. Readings were recorded as optical density at 490 nm and were expressed as the percentage of a known positive sample. Positive, negative, and blank controls (PBST) were measured for each plate. The volume in each well was 100 μl in all steps, and each sample was added in duplicate. The sample was considered positive if it exceeded mean±3 S.D. from 100 serum in normal blood donors (no clinical, autoantibody, or ultrasonographic evidence of thyroid disease).

**Statistical analysis**

Normally distributed continuous variables were expressed as means±S.D., and non-normal distributed continuous data were expressed as median (minimum, maximum). Categorical variables were summarized as frequency or percentage. Comparisons of continuous data between groups were performed by the Student’s t-test or Mann–Whitney U test and the χ² test or the Fisher’s exact test for categorical variables. Multiple binary logistic regression was used to identify the factors related to TD. Differences were considered statistically significant with a two-tailed P value <0.05. All statistical analysis was performed using SPSS 16.0 software (Statistical Package for the Social Science; SPSS, Inc.).
Results

The demographics of the 312 CHC patients enrolled in the study are shown in Table 1. As shown, 21.9% of our patients had positive TAs (TPOAb and/or TGAb) (TPOAb: 16.3%; TGAb: 13.3%). The percentage of patients that were positive for TAs was significantly higher in older patients (>60 years) (31.9 vs 18.6%, odds ratio (OR) = 2.046, 95% CI: 1.015–4.125, \(P = 0.042\)). There was no difference between males (17.8%) and females (25.5%) who had positive TAs.

Prevalence of TD

Among the 312 patients with CHC, 39 (12.5%) displayed biochemical TD in this cross-sectional study. Clinical hypothyroidism, clinical hyperthyroidism, subclinical hypothyroidism, and subclinical hyperthyroidism were seen in 18 (5.8%), five (1.6%), 12 (3.8%), and four (1.3%) of the 39 cases respectively. Table 2 shows the characteristics in CHC patients with or without TD. There was no significant difference between the two groups (43.5 vs 51 IU/l, \(Z = -1.997, P = 0.046\)).

IgG subclasses of TPOAb/TGAb

Of the CHC patients, 33 and 19 patients had detectable IgG subclasses of TPOAb and TGAb respectively. As shown in Fig. 1, IgG1, IgG2, and IgG4 were the main IgG subclasses of TPOAb in patients with TD (clinical hypothyroidism in five, clinical hyperthyroidism in two, subclinical hypothyroidism in six and subclinical hyperthyroidism in two), while IgG1 and IgG4 were the main subclasses of TPOAb in the WTD group. The percentage of TPOAb IgG2 subclass in the TD group was significantly higher than those without TD (66.7 vs 16.7%, OR = 0.1, 95% CI: 0.019–0.515, \(P = 0.005\)).

Multivariate analysis

Multiple logistic regression analysis of gender, age, TPOAb positivity, TPOAb IgG2 subclass positivity, and ALT and HCV RNA levels showed that only CHC patients with positive TPOAb IgG2 subclass were at higher risk of developing TD (OR = 8; 95% CI: 1.225–52.246, \(P = 0.030\)).

Table 1 Demographics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=312)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)(^{a})</td>
<td>48.15 ± 13.98</td>
</tr>
<tr>
<td>Gender, (% male/female)</td>
<td>49.7/50.3</td>
</tr>
<tr>
<td>Genotype, (% 1b/2a/3)</td>
<td>73.2/22.9/3.9</td>
</tr>
<tr>
<td>HCV RNA (log(_{10}) IU/ml)(^{a})</td>
<td>5.86 ± 1.12</td>
</tr>
<tr>
<td>ALT (IU/l)(^{b})</td>
<td>48.5 (8, 512)</td>
</tr>
<tr>
<td>AST (IU/l)(^{b})</td>
<td>42 (9, 310)</td>
</tr>
<tr>
<td>TBIL (μmol/l)(^{b})</td>
<td>16.3 (1, 150)</td>
</tr>
<tr>
<td>DBIL (μmol/l)(^{b})</td>
<td>4.6 (0.0, 108)</td>
</tr>
<tr>
<td>TP (g/l)(^{a})</td>
<td>74.04 ± 8.5</td>
</tr>
<tr>
<td>ALB (g/l)(^{b})</td>
<td>43 (13.2, 52)</td>
</tr>
<tr>
<td>Positive antibodies, (% TPOAb/TGAb)</td>
<td>16.3/13.3</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; TP, total protein; ALB, albumin; TPOAb, thyroid peroxidase antibody; TGAb, thyroglobulin antibody.

\(^{a}\)Means ± S.D.

\(^{b}\)Median (minimum, maximum).

Table 2 Characteristics of patients with (TD) and without (WTD) thyroid dysfunction.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TD</th>
<th>WTD</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)(^{a})</td>
<td>50.62 ± 14.82</td>
<td>47.79 ± 13.84</td>
<td>0.239</td>
</tr>
<tr>
<td>Gender, (% male/female)</td>
<td>41/59</td>
<td>50/49</td>
<td>0.248</td>
</tr>
<tr>
<td>Genotype, (% 1b/2a/3)</td>
<td>81.8/13.6/4.5</td>
<td>72.2/23.9/3.8</td>
<td>0.543</td>
</tr>
<tr>
<td>HCV RNA (log(_{10}) IU/ml)(^{a})</td>
<td>5.69 ± 1.09</td>
<td>5.88 ± 1.12</td>
<td>0.383</td>
</tr>
<tr>
<td>ALT (IU/l)(^{b})</td>
<td>43.5 (11, 258)</td>
<td>51 (8, 512)</td>
<td>0.046</td>
</tr>
<tr>
<td>AST (IU/l)(^{b})</td>
<td>32.5 (15, 197)</td>
<td>45 (9, 310)</td>
<td>0.083</td>
</tr>
<tr>
<td>TBIL (μmol/l)(^{b})</td>
<td>17.6 (4.5, 39.7)</td>
<td>15.9 (1.8, 150)</td>
<td>0.351</td>
</tr>
<tr>
<td>DBIL (μmol/l)(^{b})</td>
<td>3.94 (0.5, 11.5)</td>
<td>4.7 (0.0, 108)</td>
<td>0.461</td>
</tr>
<tr>
<td>TP (g/l)(^{a})</td>
<td>73.2 ± 10.63</td>
<td>74.15 ± 8.19</td>
<td>0.608</td>
</tr>
<tr>
<td>ALB (g/l)(^{b})</td>
<td>43.1 (13.2, 48.1)</td>
<td>43 (18.1, 52)</td>
<td>0.856</td>
</tr>
<tr>
<td>Positive TPOAb (%)</td>
<td>62.1</td>
<td>10.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Positive TGAb (%)</td>
<td>20.7</td>
<td>12.4</td>
<td>0.245</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; TP, total protein; ALB, albumin; TPOAb, thyroid peroxidase antibody; TGAb, thyroglobulin antibody.

\(^{a}\)Means ± S.D.

\(^{b}\)Median (minimum, maximum).
Discussion

In this cross-sectional study, a total of 312 CHC patients were enrolled to investigate the prevalence of TD and distribution of IgG subclasses of TAs before antiviral therapy. Previous studies have reported a significant increase in the prevalence of thyroid autoimmunity and hypothyroidism in chronic HCV-infected patients (17). A meta-analysis revealed significant associations between chronic HCV infection, thyroid autoimmunity, and hypothyroidism (18). In our study, 12.5% of CHC patients displayed TD. The frequency of overt and subclinical hypothyroidism was higher than overt and subclinical hyperthyroidism. The patients enrolled in our study (TD and WTD groups) had similar distributions by sex, age, and virological indicators (HCV genotype and viral load). It has been reported that lower ALT and increased TPOAb levels were more commonly found in CHC patients with TD than those without thyroid disorders (19, 20). We observed similar trends between TD and euthyroid patients.

Previous reports showed that the incidence of TAs in HCV-infected patients ranged from 10 to 45% (21, 22, 23). These observed differences might be related to geographical distribution, genetic variability, and environmental factors (24). In our study, 21.9% patients had positive TAs, with positive TPOAb and TGAb being found in 16.3 and 13.3% of the patients respectively. Recent studies suggested that females carried a higher risk of TAs than male patients with risk increasing with age (25). Our data showed that TAs were more common in patients that were older than 60 years. The prevalence of TAs in female patients with chronic HCV infection was higher but was not significant compared with male patients. This observation was consistent with previous studies, which reported that there was no difference between females and males with respect to TAs before treatment (20, 26).

TPOAb/TGAb was not restricted to any particular isotype but comprised of all four IgG subclasses in our study: IgG1, IgG2, IgG3, and IgG4. Each IgG subclass possesses different biological functions. Protein antigens mainly induce IgG1 and IgG3 or IgG1 and IgG4, while the response to polysaccharide antigens are restricted in most cases to IgG2 (27). IgG1 and IgG3 are more capable of binding to monocytes and neutrophils and activating the complement system than IgG2 and IgG4 (28). IgG1 and IgG3 have the highest affinity for C1q and the strongest antibody-dependent cell-mediated cytotoxicity (ADCC), while IgG2 is less capable of fixing complement and mediating ADCC (29, 30, 31). IgG4 is a poor mediator of complement fixation and ADCC (31, 32) and is a marker of chronic antigen exposure (33). Kotani et al. (34) reported that TPOAb consisted of mainly IgG1 subclass in patients with autoimmune thyroid diseases. Parkes et al. (35) observed that TGAb was predominantly found in, but not exclusively associated with, IgG4 subclass in patients with autoimmune thyroiditis. Consistently, the predominant subclasses of TPOAb and TGAb in our TD group were IgG1 and IgG4 respectively. In the WTD group, the predominant subclasses of TPOAb and TGAb were both IgG1. IgG1 is important during inflammation (36) and presents regardless of thyroid function (37). Higher IgG4 levels are detectable with prolonged immune response during injury (38).

In this study, the prevalence of TPOAb IgG2 subclass in the TD group (66.7%) was significantly higher than that of patients without TD (16.7%). Multiple logistic regression analysis also indicated that TPOAb IgG2 subclass was the only factor that correlated with the presence of TD in CHC patients. The correlation of TPOAb IgG2 subclass with TD seems not to be specific in CHC patients. Our previous study on 168 newly diagnosed Hashimoto’s thyroiditis patients demonstrated that the prevalence of TPOAb IgG2 subclass was significantly higher in hypothyroidism (51.5%) and subclinical hypothyroidism (33.3%) than in euthyroidism (11.9%) (39). Weetman et al. (40) observed that
IgG2 had the highest relative functional affinity and differed in effector function from IgG1 and IgG4 thyroid antibodies. IgG2 expression is induced by IFN-gamma (IFN-\(\gamma\)) (41). Chemokine ligand 10 (CXCL10) can induce the recruitment of T helper (Th) lymphocytes, which secrete IFN-\(\gamma\) (42). It is reported that serum CXCL10 levels were significantly increased in CHC patients when compared with sex- and age-matched healthy volunteers (43). This might explain the significantly higher prevalence of TPOAb IgG2 subclass observed in TD patients in our study.

Altogether, our cross-sectional study showed that CHC patients with TD before IFN treatment had higher TPOAb levels and lower ALT levels than euthyroid ones. We identified TPOAb IgG2 subclass positivity as a risk factor for TD in CHC patients. IgG2 subclass of TPOAb might play an important role for TD in CHC patients. To our knowledge, this is the first study to investigate the distributions of IgG subclasses of TPOAb and TGAbs in CHC patients with or without TD. Additional studies are needed to fully elucidate the mechanisms involved in HCV-related TD.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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