

Thyroid disorders in patients with chronic hepatitis C using interferon-alpha and ribavirin therapy

ABSTRACT

Objective: To investigate the frequency of thyroid disorders (TD) in patients with chronic hepatitis C before and during interferon-alpha (IFN- α) and ribavirin (RIB) treatment. **Study design:** Prospective study. **Patients and Methods:** We prospectively studied 65 anti-HCV and viral RNA positive patients. Free thyroxine, thyroid-stimulating hormone, and thyroid peroxidase antibodies (TPO-Ab) were systematically tested at entry (m0), week 12 (m3) and week 24 (m6) of treatment. **Results:** Mean age of the 65 patients (38 females and 27 males) was 49.61 ± 11.83 years. Seven (10.76%) patients presented baseline thyroid disorders (m0), three had thyroid dysfunction, and four were TPO-Ab positive. Thyroid disorders occurred in the first 12 weeks of treatment in 11 (16.92%) patients, four with thyroid dysfunction, and seven with TPO-Ab positive (m3). A total of 18 patients (27.69%) developed TD after 24 weeks of treatment, 7 with thyroid dysfunction, and 11 with TPO-Ab positive (m6). The relative risk of developing hypothyroidism found in this study was 1.3 (95% CI: 1.1 to 1.6), hyperthyroidism 1.2 (95% CI: 1.1 to 1.4), and TPO-Ab positivity 7.6 (95% CI: 3.9 to 14.5). The study showed a significant association between female sex and thyroid disease ($p = 0.009$). **Conclusion:** Thyroid dysfunction and autoimmune TD were observed during IFN- α and RIB therapy.

Keywords: hepatitis C; interferon-alpha; ribavirin; thyroid diseases; autoimmunity.

INTRODUCTION

Thyroid dysfunction represents one of the commonest endocrine manifestations of chronic hepatitis C infection (HCV), exacerbated by interferon-based treatment.¹ Changes in thyroid function are common side-effects occurring during antiviral therapy with interferon-alpha (IFN- α). In this way, the spectrum of thyroid diseases range from the production of isolated anti-thyroid antibodies to dysfunctions such as hypothyroidism, Graves' disease (GD), and destructive thyroiditis.²

IFN- α therapy for HCV may induce thyroid changes or dysfunction in 2.5% to 20% of treated patients. Up to 40% become thyroid antibody positive, and these side-effects can interfere with effective management of HCV.^{3,4}

Almost all side-effects of IFN- α treatment are due to its effects on the immune system, and data suggest that in addition to its immunomodulatory mechanism, IFN- α also precipitates thyroiditis by direct thyrotoxicity.⁴

Ribavirin (RIB) is a nucleoside analogue with broad spectrum activity against several RNA and DNA viruses, and it could possibly stimulate the immune system alone or synergistically with IFN- α to cause thyroid disease via an autoimmune mechanism.⁵ RIB can also enhance non-virus-induced immune response, suggesting that this drug could trigger autoimmune phenomena in predisposed patients.⁶ Thyroid dysfunction is more prevalent in patients treated with IFN- α and RIB combination therapy (12.1%) than in patients treated with IFN- α alone (6.6%).⁷

Additionally, hepatitis C virus is both a hepatotropic as well as a lymphotropic virus and can modulate T cell and B cell antibody responses, affecting most commonly the thyroid.^{8,9} However, morphological evidence of hepatitis C virus replication in thyroid cells in immune competent patients has not been demonstrated.¹⁰ The objective of this study was to assess the frequency of

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thyroid disorders in patients with HCV before and during long term IFN- α and RIB therapy, and to assess the relative risk of dysthyroidism in this population.

MATERIALS AND METHODS

Study design

This prospective study was conducted at the Gastro-Hepatology Unit, *Universidade Federal da Bahia*, Salvador, Brazil, and all patients gave their informed consent to participate. The study was approved by the ethical committee of *Universidade Estadual de Santa Cruz*, Bahia, Brazil, in accordance to the Declaration of Helsinki.

Thyroid disease was investigated in all patients by tests that included thyroid stimulating hormone (TSH), free thyroxine (FT4), and antithyroid peroxidase antibody (TPO-Ab) titers, before initiating treatment (m0), at week 12 (m3) and at the end week 24 of treatment (m6). Other autoimmune diseases were not investigated in this study. Thyrotropin receptor antibodies (TRAb) are useful in the diagnosis of GD disease. Measurements of TRAb were only performed in patients who had clinical hyperthyroidism to assess whether the GD was active.

Study patients

Our study included 65 patients admitted for HCV infection to the Gastro-Hepatology Unit, *Universidade Federal da Bahia*, Salvador, Brazil. The eligibility criteria were: I) serum anti-HCV antibody and HCV RNA positivity; II) chronic inflammation confirmed by histological analysis of the liver; III) available genotyping; and IV) treatment-naïve patients.

Virological assays

Detection of antibodies to HCV (anti-HCV) was measured by enzyme-linked immunosorbent assay, using structural and non-structural HCV antigens (AXSYM System; Abbott Laboratories, Chicago, IL, USA). HCV-RNA were quantified by polymerase chain reaction (RT-PCR) using primers derived from the highly conserved region 5'UTR (untranslated region) of the viral genome (Amplikor[®] HCV Detection KIT V2.0; Roche Molecular Systems Inc., Somerville, NJ, USA). HCV genotyping was performed by INNOLIPA (Innogenetics, NV, Gent, Belgium). Genotypes were classified according to Simmonds et al.¹¹

Liver histopathology

All subjects positive for HCV-RNA underwent percutaneous liver biopsy, obtained blindly (Hepafix 1.4 mm, B. Braun, Germany), and histological parameters were classified according to the score system of inflammation activity and fibrosis. Liver biopsy specimens were obtained before initiation of therapy and were evaluated by a single liver pathologist with expertise and blinded to the study design.

Histological abnormalities of liver biopsies were scored according to the METAVIR system. Liver fibrosis was staged on a scale of 0 - 4, here 0 = no fibrosis, 1 = portal fibrosis without septa, 2 = few septa, 3 = numerous septa without cirrhosis, and 4 = cirrhosis. Necroinflammatory activity was graded on a scale of A0-A3, where A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.^{12,13}

Evaluation of thyroid function and antibody thyroid screening

TSH (ultrasensitive third-generation method with a reference normal range of 0.35-5.50 mIU/L), and FT4 (reference normal range of 0.58-1.40 ng/dL) were assayed using commercially available kits by immunometric assays.

TPO-Ab was detected by solid phase 2-site sequential chemiluminescent immunometric assay (normal: < 40.0 IU/mL). Patients were classified as positive (TPO-Ab > 40.0 IU/mL) or negative (TPO-Ab > 40.0 IU/mL) for thyroid autoimmunity.

Study outcomes

The primary outcome of this study was the development of thyroid dysfunction during IFN- α and RIB therapy. Secondary outcomes included frequency of TPO-Ab at the end week 24 of HCV therapy. The association between viral load and sustained virological response (SVR) with the development of thyroid disorders was not assessed in this study protocol.

Statistical analysis

Data are expressed as mean \pm SD for those variables that were normally distributed. Statistical analysis was performed using chi-square or Fischer's exact test. Relative risk was calculated by using the one-tailed exact method for the combination of 2 \times 2 tables, computing 95% confidence intervals (CI).

Statistical analysis was performed using SPSS[®] version 16.0 for Windows (SPSS Inc, Chicago, Ill), and a p-value < 0.05 was considered statistically significant.

RESULTS

Mean age of the 65 patients (38 females and 27 males) was 49.61 \pm 11.83 years (range: 22-66 years). Other major characteristics of the 65 studied patients before treatment are shown in Table 1.

Table 1. Characteristics of the study patients

Characteristics	Total
Genotype (1/2/3)	46/1/18
Stage of fibrosis (1/2/3/4)	7/37/16/5
Necroinflammatory activity (0/1/2/3)	13/20/30/2

Thyroid function and TPO-Ab before treatment

Before treatment (m0), thyroid function tests and TPO-Ab titers were evaluated in all patients. Seven (10.76%) patients presented baseline thyroid disorders, one individual had overt hypothyroidism, one subclinical hypothyroidism, one subclinical hyperthyroidism, and four were positive for TPO-Ab.

Titers of positive TPO-Ab cases were similar for females and males (about 3.07%). The positivity of TPO-Ab before treatment was more commonly found in patients with thyroid dysfunction than those without thyroid disorders ($p = 0.04$). No significant association was found among sex, age, HCV genotypes, or METAVIR with thyroid disorders at baseline. Patients who had thyroid dysfunction were treated before the start of therapy with IFN- α and RIB.

Thyroid function and TPO-Ab at week 12 of treatment

After 12 weeks of treatment (m3), two patients (3.07%) developed subclinical hypothyroidism *de novo*, one patient (1.54%) developed clinically overt hyperthyroidism *de novo*, and three patients (4.62%) presented positive TPO-Ab titers *de novo*. One case of subclinical hypothyroidism *de novo* occurred during treatment was associated with TPO-Ab positivity. Statistical analysis showed significant correlation between the development *de novo* thyroid dysfunction during IFN- α and RIB treatment and TPO-Ab positivity at week 12 of treatment ($p = 0.001$). Out of 11 cases with thyroid disorders at the end of the 12th week of treatment (16.92%), four cases had thyroid dysfunction and seven had positive TPO-Ab, including baseline and *de novo* cases. Patients who had thyroid dysfunction were treated upon diagnosis.

Thyroid function and TPO-Ab at the end of week 24 of treatment

At the end of 24 weeks of treatment (m6), three (4.62%) patients developed thyroid dysfunction (two subclinical hypothyroidism, and one subclinical hyperthyroidism

de novo), and four patients (6.15%) presented positive TPO-Ab titers *de novo*. No case of thyroid dysfunction *de novo* occurred during treatment associated with TPO-Ab positivity assessed at week 24 of treatment. Therefore, 18 cases of thyroid disorders occurred at the end of 24 weeks of treatment (27.69%), being seven cases of thyroid dysfunction, four individuals had subclinical hypothyroidism, and three subclinical hyperthyroidism ($p = 0.006$). Additionally, 11 cases of positive TPO-Ab were diagnosed including baseline, 12 weeks of treatment, and *de novo* cases (Table 2).

The relative risk (RR) of developing thyroid autoimmunity was significantly higher among those who developed thyroid dysfunction. It was found that the RR of developing hypothyroidism in this study was 1.3 (95% CI 1.1 to 1.6; $p = 0.02$), of hyperthyroidism was 1.2 (95% CI 1.1 to 1.4; $p = 0.02$), and of TPO-Ab positivity was 7.6 (95% CI 3.9 to 14.5; $p = 0.001$). The RR of developing thyroid autoimmunity in HCV carriers treated with IFN- α and RIB was 5.8 times higher than the risk for developing thyroid disorders.

The thyroid disorders occurred more often in female patients aged over 40 years old, with a significant association ($p = 0.009$) when analyzed after 12 weeks of therapy. The association between viral load, and SVR with the development of thyroid disorders at the end of the m6 was not assessed.

DISCUSSION

This study reports the frequency of thyroid disorders (thyroid dysfunction and thyroid autoimmunity) before and during antiviral combination therapy with IFN- α and RIB for HCV. The frequency of thyroid disease at the end of 24 weeks of antiviral therapy with IFN- α and RIB therapy for HCV were 27.69% in our study (10.77% and 16.92% for thyroid dysfunction and TPO-Ab positivity respectively).

Table 2. Thyroid function test before, at week 12, and at week 24 of treatment

	Thyroid function before treatment				Thyroid function 12 weeks of treatment				Thyroid function 24 weeks of treatment			
	Normal (%)	Anormal (%)	Positive (%)	Negative (%)	Normal (%)	Anormal (%)	Positive (%)	Negative (%)	Normal (%)	Anormal (%)	Positive (%)	Negative (%)
FT4	64 (98.46)	1 (1.54)			64 (98.46)	1 (1.54)			64 (98.46)	1 (1.54)		
TSH	62 (95.38)	3 (4.62)			60 (92.30)	5 (7.70)**			60 (89.23)	7 (10.77)****		
TIPO-Ab			4 (6.15)*	61 (93.85)			7 (10.77)***	58 (89.23)			11 (16.92)*****	54 (83.08)

* $p = 0.04$; ** $p = 0.0001$; *** $p = 0.0001$; **** $p = 0.0006$; ***** $p = 0.08$.

Our series demonstrates a wide spectrum of thyroid diseases, ranging from overt hypothyroidism and hyperthyroidism, and thyroiditis until subclinical hypothyroidism or hyperthyroidism. These findings are in agreement with previous studies reporting thyroid dysfunction between 2.5% and 20%, with a mean incidence of 12.1%, and up to 40% of patients becoming thyroid antibody positive under therapy with IFN- α and RIB.^{3,4}

Several extrahepatic diseases have been associated with HCV, but thyroid disorders are the most common endocrinopathy, exacerbated by IFN- α and RIB treatment.¹⁴ IFN-induced thyroid disease exhibits features from both drug-induced thyroiditis and autoimmune thyroid disease. However, the mechanisms of induced thyroid disease are complex and not completely understood. The clear association between autoimmune thyroid disease and IFN- α use suggests that high endogenous IFN- α levels may also be associated with naturally autoimmune thyroid disease.¹⁵ RIB is a nucleoside analogue with broad spectrum activity against several RNA and DNA viruses with immunomodulatory effects. It is possible that RIB may stimulate the immune system alone or synergistically with IFN- α to cause thyroid disease via an autoimmune mechanism.¹⁶

IFN-induced autoimmune thyroid disease is associated with elevated auto-antibodies and may manifest with or without clinical disease.¹⁷ The presence of TPO-AB prior to IFN- α therapy carries a relative risk of 3.9 (95% CI 1.9-8.1) for thyroid disease.¹⁵ Ours results showed an increased frequency of TPO-Ab positivity after initiation of therapy with IFN- α and RIB. The relative risk of developing thyroid autoimmunity among HCV carriers treated with IFN- α and RIB was 5.8 times higher than the risk for developing hypothyroidism or hyperthyroidism.

Recent biochemical advances have led to the development of highly sensitive assays of serum TSH and FT4 for the biochemical assessment of thyroid function. TSH is the most sensitive marker of thyroid function, its determination with these new tools makes it possible to detect both subclinical hypo- and hyperthyroidism (elevated or decreased TSH, with normal FT4, respectively). The rate of subclinical forms of thyroid dysfunction is significantly higher in patients treated with IFN- α for HCV.¹⁸ This study showed a higher frequency of subclinical thyroid dysfunction. These findings have been found by other investigators and suggest that combination therapy for HCV can be continued, even in those who develop overt thyroid disease.¹⁹

IFN- α can trigger the development of hyperthyroidism in predisposed individuals.²⁰ In previous studies, the incidence of hyperthyroidism during IFN- α -based therapy was 1-2%.²¹ In this study our data is in accordance with the literature, where one case developed hyperthyroidism

following IFN- α therapy and did not recover after the end of the antiviral therapy.

The strongest risk factors that were associated with an increased risk of development of thyroid disease during IFN- α therapy were female gender and the presence of TPO-Ab before the initiation of therapy. In this way, the risk among females increases directly with increasing age.^{15,22} Our study showed a significant association between female gender with age over 40 years old and thyroid disease.

Rodríguez-Torres et al.²³ demonstrated that patients with HCV and severe fibrosis have higher probability of prior history of thyroid disorders and are more prone to develop thyroid disorders events during treatment with IFN- α than patients with mild fibrosis. Our study showed no significant differences between thyroid dysfunction patients and liver inflammation or fibrosis grade, but it should be pointed out that 89.2% of patients presented a stage of fibrosis equal to or above F2.

Significant association between thyroid disease and SVR was described by Tran et al.¹⁴, which was not supported by meta-analysis. The association between viral load, and SVR with the development of thyroid disorders was not part of this study protocol, considering that our primary objective was the development of thyroid dysfunction during IFN- α and RIB therapy, and the secondary objective was frequency of TPO-Ab at the end of 24 weeks of HCV therapy. Despite the lack of supportive evidence from meta-analysis, it is important that future studies be done to confirm or reject these results.

CONCLUSION

In conclusion, this study shows important clarification of the association between HCV treatment and thyroid disorders. Accordingly, some guidelines may be suggested as follows: patients who are treated with IFN- α and RIB therapy should be informed about the risks of developing thyroid dysfunction, and therefore screening of thyroid function and the panel of antibodies should be evaluated before and during treatment of HCV.

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