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Original Article

Thyroid dysfunction (TD) among chronic hepatitis C patients with mild and severe hepatic fibrosis

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Abstract

Background: **Thyroid dysfunction (TD) is associated to chronic hepatitis C (HCV) and interferon (IFN) therapy. The prevalence of TD at baseline and during IFN therapy among stages of hepatic fibrosis is unknown.** ***Goals*****: To examine the frequency of TD at baseline and during Peg-IFN therapy among patients with severe and mild fibrosis.** ***Study:*** **100 patients were treated with Peg-IFN and divided in 2 groups (50 each), according to liver histology; Metavir 0-2 (mild fibrosis) and Metavir 3-4 (severe fibrosis). Baseline TD was defined as history of TD, or abnormal thyroid stimulating hormone (TSH) or antiperoxidase thyroid auto-antibodies (TPO -Ab). Frequency of TD during therapy was defined as TD that required treatment.** ***Results*****: 20% in the severe fibrosis group and 10% in the mild fibrosis group, had TD at baseline. Most of the cases, 31.4% were female as compared to 6.25% males. During therapy, 24% of patients in the severe fibrosis group, compared to 12% in the mild fibrosis, had TD. Most patients had biochemical hypothyroidism, and 66% were female, compared to 33.33 % male. TPO-Ab predicted TD during therapy in 50% of cases while those negative only had 16.6% TD during IFN therapy.** ***Conclusions:*** **Patients with severe fibrosis have more TD events at baseline and during treatment with Peg IFN alfa-2a. Patients with more hepatic fibrosis require careful attention to diagnose and manage TD. More research in the immune mechanisms of hepatic fibrosis progression and autoimmune complications is needed.**

Key words: Autoimmune thyroid dysfunction, HCV, fibrosis.

Background

Autoimmune disorders have been described in association with chronic hepatitis C infection (HCV), including thyroid disease, including hypothyroidism, sialodenitis, autoantibody formation and autoimmune idiopathic thrombocytopenic purpura.[1-3,4] Autoimmune thyroid dysfunction (TD) is common,[2,5-10] and reported to be more frequent in women.[11] Overall, anti thyroid antibodies are present in 5-17% of patients with HCV infection and TD occurs in 2-13% of patients.[2,10] A higher prevalence in older women has been reported but this finding is controversial.[12,13]

It is also known that treatment with IFN induces TD, mostly biochemical and asymptomatic in 1 to 5% of cases.[12-15] More recent reports have shown TD in 3-15% of cases with various clinical manifestations.[3,5-18]

TD during treatment is not related to IFN dosage or response to treatment.[17] There have been reports suggesting that prevalence of TD and is similar among responders and non-responders to IFN therapy and not related to hepatic histology after HCV treatment.[18] The findings in this study however are questionable because it compared histology after treatment, and patients that achieve sustained viral response will have marked improvement compared to patients that did not respond to treatment (SVR).[18]

To our knowledge, the prevalence of TD markers before treatment and the incidence of biochemical or clinical TD throughout treatment with Peg-IFN Alfa among cohorts with mild or severe fibrosis have not been reported. A clinical trial that offered treatment to patients with chronic hepatitis C and stratified patients according to severity of fibrosis by centrally reviewed hepatic histology provided the opportunity to perform an exploratory analysis of TD along fibrosis staging.

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Endpoints

Primary endpoints were to examine the occurrence of TD at screening among CHC patients and to assess the frequency of TD at any time during treatment with Peg IFN alfa-2a among CHC patients with mild and severe fibrosis. Secondary endpoint was to assess the status of clinical or biochemical TD at last scheduled follow up among CHC patients with mild or severe fibrosis.

Methods

We studied 100 consecutive subjects that were enrolled in a clinical study for HCV patients that were non-responders (NR) to prior treatment with IFN or IFN/RBV combination. For enrollment, patients were required to be null non responders, (decrease of less than 1 log from baseline) to IFN based therapies that lasted at least 12 weeks. Patients were required to have completed prior treatment at least 6 months before baseline. Patients were enrolled in (2) distinct study groups, differentiated solely on basis of hepatic biopsy staging at screening, interpreted centrally by an expert histo-pathologist. Liver biopsies were performed under CT guidance with an 18 gauge thru cut needle and gun, by an experienced invasive radiologist. All biopsies were required to be more than 25 mm long and to have more than 12 portal spaces. Patients in Group A were those with mild fibrosis defined as Metavir score 0-2 (equivalent to Ishak 0-3), and patients in Group B had severe fibrosis defined as Metavir 3-4 (equivalent to Ishak 4-6).[19] Inclusion and exclusion criteria were the same for both groups except for fibrosis staging. Patients had multiple safety and efficacy assessments at screening and at multiple time points during the treatment duration of 12 months and 6 months after last dose of drug (M-18). The assessments included HCV RNA, chemistry and blood counts and the following thyroid function tests: thyroid stimulating hormone (TSH), anti-peroxidase thyroid autoantibodies (TPO-Ab) and thyroid hormone levels. Patients with history of thyroid disease and in treatment were allowed in the study if thyroid function was normal at entry. If TSH abnormality was found at screening, documentation of normalization was required by repeat testing prior to enrollment. Laboratory assessments for all patients were performed at a central laboratory (Lab Corp, NC). All patients received same IFN treatment, Peg IFN alfa-2a (Pegasys) 180 mcg sc weekly and thymalfasin /placebo three times per week, for 48 weeks (0.8 mg, 1.6 mg and 3.2 mg). The study was sponsored by Sciclone Pharmaceuticals (San Mateo, CA, IND #37,110) and the study results were reported elsewhere.[20]

Definitions

Baseline TD markers were defined as past medical history of thyroid disease with or without treatment, abnormal TSH (range 0.350 to 5.50 mIU/mL) or high TPO-Ab (> 34 IU/mL) at screening. The number of patients with at least one TD marker were added.

TD during therapy was defined as TSH abnormality at any time without (biochemical) or with symptoms (clinical) that required treatment. Patients were started in treatment if TSH abnormality was increasing in repeated assessments or if patient had any clinical symptom.

TD at follow up was defined as any TSH abnormality or patients receiving thyroid replacement at last follow up. (6 months after end of therapy).

All patients signed informed consent for participation in this study, and agreed for confidential medical information to be analyzed. The University of Puerto Rico-School of Medicine IRB approved this study.

Statistics

As this was an exploratory retrospective study, ad hoc analysis was performed. Baseline demographic and disease specific variables were examined by mean and median. Numbers of events were reported as % of patients in each group. Fisher's exact test was used to compare variables when possible. A p value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics *(Table I)*

The patients of both Groups are similar in age, sex, and HCV characteristics (all genotype 1, and similar HCV RNA PCR). Liver biopsy showed no significant differences in grading score (necrosis, inflammation) or hepatic steatosis between the groups. As per protocol, there is a significant difference in staging (fibrosis) with Group A (mild fibrosis) mean Ishak score of 2.43 compared to Group B, (severe fibrosis) mean Ishak score of 5.02 (transition to cirrhosis).[21] Most patients in each Group were prior non responders to IFN mono-therapy (60%), compared to 40% NR to IFN/Ribavirin (RBV) combination. In Group A, 46% of cases received placebo instead of thymalfasin compared to 50% in Group B. In both groups, the majority of patients had completed prior IFN based therapy, 1.5 to 2.0 years before baseline.

Thyroid dysfunction markers at screen and during IFN therapy *(Table II)*

At screening, 10/50 (20%) of patients in group B had TD, compared to 5/50 (10%) in group A (p = 0.161). The number of patients with TPO-Ab, or TSH abnormalities was similar in both Groups. Only 2 patients in each group had high TPO-Ab at screening and none had prior history of thyroid disease. One of each, or ½ (50%), developed hypothyroidism during treatment and required re-

placement therapy. Among patients with normal TPO-Ab at screen 5/48 (10.4%) of patients in Group A and 11/48 (22.9%) in Group B developed TD during IFN therapy. For both groups the predictability of high TPO-Ab for TD during therapy was 2/4 (50%) compared to only16/96 (16.6%) for those with normal TPO-Ab levels. This difference did not reach significance probably because low number of patients (p = 0.089).

Summary of patients that required treatment for TD *(Table III)*

Table III shows all patients treated for TD during study and status at last follow up.

During HCV therapy a higher number of patients in Group B, 12 patients required treatment for TD, all for hypothyroidism, compared to 6 in Group A, (p = 0.12), including one (1) patient with symptomatic Grave's disease. Most patients with hypothyroidism were asymptomatic, except 2 patients in Group B. A review of the clinical course of TD suggests that 3 cases in group A had classical biphasic thyroiditis with resultant hypothyroidism. No case in Group B had a similar course.

The only patient with symptomatic TD in Group A was the patient with Graves' disease and required subtotal thyroid resection for management. The pathology review of the surgical specimen revealed Papillary Carcinoma and patient had total thyroid removal. Patient remains stable and free of tumor three years later.

Most patients that developed biochemical hypothyroidism in Group A had TSH abnormalities late in therapy with 4/6 after 6 months. However, patients in group B developed TSH abnormalities earlier, 8/12, within 3 months and almost all cases within 6 months of therapy (10/12). The only case that had clinical hyperthyroidism (Group A) had initial changes in TSH at month 8, but clinical symptoms occurred at end of therapy. The large majority of patients had normal TSH at last follow up, but most continued in thyroid hormone replacement.

Table I. Baseline characteristics.

Characteristic	Group A (n = 50)	Group B (n = 50)	p
Sex F/M	17/33 (66%)	18/32 (64%)	0.927
Age (years)			
Mean	48.71	56.72	0.816
Median	50.00	58.00	
Ishak Score			
Grading			
Mean	4.45	7.66	0.095
Median	5.00	7.00	0.701
Staging			
Mean	2.43	5.02	< 0.01
Median	3.00	5.00	
Liver steatosis > 5%	6 (12%)	6 (12%)	1.000

Group A (mild fibrosis) and Group B (severe fibrosis) are similar in gender, age grading and steatosis. The cohorts only differ only in stage of fibrosis.

Gender differences

Females had more TD markers at screening compared to males in both groups; Group A, p = 0.196 and Group B, p = 0.001, for a total of females with positive markers of 11/35 compared to males 4/65 (p = 0.001). Most of the patients that required treatment for TD during the study were females, 5/6 in Group A and 7/12 in Group B for a total of 12/18 p = 0.04 *(Table II)*. Out of 17 females (34%) in group A and 18 females (36%) in group B, only 2/35 (5.7%) had abnormal TPO-Ab levels at screening. There was a female patient in group A with normal TPO-Ab and TSH at baseline who developed high TPO-Ab

Table II. Thyroid dysfunction at screening, during Peg IFN therapy.

Characteristic	Group A (n = 50)		Group B (n = 50)		Total (n = 100)	
Thyroid markers at baseline						
Sex F/M	17/33		18/32		35/65	
Hx Thyroid disease	2		7		9	
TSH > 5.500	1		1		2	
TSH < 0.350	0		1*		1	
TPO-AB	2		2		4	
Total	5		10	p = 0.161	15	
Female n/%	3 (17)/17.6	p = 0.195	8 (18)/44.4	p=0.001	11/35	p = 0.0007
Male n/%	2 (33)/6.6		2 (32)/6.2		4/65	
TPO-AB Predictability of TD during tx						
Positive n/%	1 (2)/50	p = 0.091	1 (2)/50	p=0.379	2 (4)/50	p=0.089
Negative n/%	5 (48)/10.4		11 (48)/22.9		16 (96)/16.6	
TD requiring tx and gender	6 (12%)		12 (24%)			
Females n/%	5 (6)/83.3	p=0.020	7 (12)/58.33	p=0.414	66.66	
Males n/%	1 (6)/16.6		5 (12)/45.14		33.33	p = 0.045

* Same patient had history and abnormal TSH.

Most of the patients with TD at screening are females (p 0 0007). More females than males in Group B require treatment than males (p = 0.001). High TPO Ab at screen predict TD during Peg IFN therapy in 50% of cases, compared to 16.6% in patients with normal levels at screen (p = 0.089). Group A patients that required therapy were 6/50 or 12% compared to 12/50, or 24% in Group B. The difference along gender was significant, p = 0.045.

Table III. Summary of patients that required therapy for TD.

Group A (n = 6)								
Sex F/M	Prior Hx	Time of TSH alteration	C/B		TPO Ab		End of F-U	
				BL	EOT	F-U	TSH N/A	TX
Male	No	M-10 (TSH↑ 18.085)	B	43	2,242	484	N	Yes
Female	No	M-8 (TSH↑ 9.85)	B	< 10	13	12	N/A	N/A
Female	No	M-9 (TSH↑ 35.5)	B	< 10	10	13	N	Yes
Female	No	Screen (TSH↑ 6.7)	B	17	14	18	N/A	N/A
Female	Yes	M-3 (TSH↑ 7.8)	B	13	16	22	A↓	No
Female	Yes	M-6 (TSH↓ 0.197)	C	10	28	10	A↓	No
Group B (n=12)								
Sex F/M	Prior Hx	Time of TSH alteration	C/B		TPO Ab		End of F-U	
				BL	EOT	F-U	TSH N/A	TX
Female	No	M-6 (TSH↑ 9.535)	B	10	20	15	N	Yes
Male	No	M-6 (TSH↑ 6.62)	B	< 10	15	11	N	Yes
Male	Yes	Replacement (TSH normal)	B	22	82	49	N	Yes
Female	Yes	M-9 (TSH↑ 75.6)	C	< 10	15	11	N	Yes
Female	Yes	M-3 (TSH↑ 7.851)	B	14	10	N/A	N/A	N/A
Male	No	M-3 (TSH↑ 8.63)	B	27	18	< 10	A↑	No
Female	No	M-3 (TSH↑ 7.28)	B	16	28	15	N/A	N/A
Male	Yes	M-3 (TSH↑ 10.206)	B	10	13	11	N/A	N/A
Male	No	M-3 (ET) (TSH↑ 6.79)	B	10	10	10	N	Yes
Female	Yes	Replacement (TSH↑ normal)	B	22	82	49	N	Yes
Female	Yes	Replacement (TSH↑ normal)	B	10	10	22	N	Yes
Male	No	M-9 (TSH↑ 92.45)	C	81	205	70	N	Yes

Table shows information for all cases that required TD therapy, including gender, prior history of thyroid disease, time point of TSH alteration, TPO Ab results at baseline, end of treatment (EOT) and last follow up (F-U) and therapy. Table also shows status of all cases at end of F-U.

without TSH abnormality during treatment or at last follow up. A male patient at Group B had the same clinical course.

Discussion

We have demonstrated that patients with CHC and severe fibrosis have higher probability of prior history of TD and develop more frequently TD events during treatment with Peg-IFN Alfa-2a than patients with mild fibrosis. The number of TD markers at baseline in the patients with severe fibrosis is among the higher reported in the medical literature. This suggests a relationship between liver damage (fibrosis) progression in chronic hepatitis C and the risk for TD.

The association of autoimmune diseases with chronic hepatitis C has been reported extensively.[1-13,22] It has been postulated that the pathogenesis of these diseases involve genetic and environmental factors.[5] Some studies have reported association between non organ specific auto antibodies (NOSA) and the risk for autoimmune thyroid dysfunction.[23] The best predictor was found to be LKM 1 antibody for both de novo autoimmune thyroid markers and symptomatic TD. Unfortunately, we only performed TPO-Ab in this study and although the number of patients was small, 50% of patients with TPO-Ab required TD treatment during PegIFN therapy, as has been reported.[24] The difference in prior history of TD at baseline may suggest that immune processes that affect progression of liver disease, may be also associated to increased risk of TD.

TD is also a known adverse event or complication during IFN treatment. It has been reported to be more frequent in females, and to be mostly hypothyroidism (both clinical and biochemical) and to resolve after end of therapy.[1-13,24,25] In our study, the majority of TD events at screen and during Peg IFN therapy occurred in females, as has been reported.[26-28] IFN therapy induces a surge of immune reactions in the body. IFN binds to specific receptors on the cell surfaces initiating intracellular signaling via a very complex sequence of protein-protein interactions and gene transcription activation.[29-31] IFN effects in peripheral lymphocytes, specifically patients that develop Type 1 immune responses have been associated to TD whereas those that remain euthyroid have mostly Type 2 immune responses.[32] It is unlikely that the cohorts in this study had any residual effect of prior IFN therapy as it was completed 1.5 to 2 years before enrollment. The number of patients that developed thyroid disease during therapy was almost equally divided among those that received placebo and those that received thymalfasin, excluding an affect of randomization. Thymalfasin has not been associated to TD, so is further improbable this affected the number in events in each group. In our cohort, the only difference between the groups was the fibrosis score. The cohorts were not significantly different in age,

sex and from previously reported data on our population, we estimate a similar risk for HCV infection and duration of infection.[33] The differences in progression to cirrhosis among patients with HCV have been widely reported.[33-35] We may postulate that differences in immune pathways activation may differ among patients that have slow and rapid progression, and also impact the risk for autoimmune diseases related to HCV. This would also be congruent with our finding of higher and earlier occurrence of TD in the severe fibrosis group. We can not exclude genetic factors to play an important role in risk for TD in our cohort as an association with the HLA system of locus A, B and C and alleles of locus DRBI and DQBI has been reported for patients that develop TD during IFN therapy.[36] Although a more rapid progression to cirrhosis in Latinos has been reported,[33] it is unknown if Latino ethnicity is a predictor of HCV related autoimmune disease. The number of TPO-Ab positive patients at baseline was lower than reported in other studies, however, the predictability of TD during IFN therapy for patients with high TPO-Ab levels was similar to a recent report.[37]

In this study we documented a case of Thyroid Papillary Carcinoma, considered to be associated to HCV and other related immune disorders as cryoglobulinemia.[38-40] In this setting, thyroid carcinoma may be the more severe manifestation of TD.

This study has limitations. The cohort is small and is a retrospective appraisal of a clinical trial data with the inherent bias of patient selection. However as an exploratory evaluation of TD differences along severity of liver fibrosis, the results are still clinically relevant. The findings at baseline are not reliable prevalence data, and require corroboration in prospective epidemiological surveys with larger number of patients. It is of notice however, that our observation of more TD markers in the severe fibrosis group at screen is concordant with the higher frequency of TD therapy during IFN treatment. Prospective studies with larger number of patients are necessary to determine prevalence and incidence of TD along fibrosis stages.

In summary, this study suggests that patients with severe fibrosis are more likely to have TD and to require treatment for TD during therapy with Peg IFN than those with mild fibrosis. We postulate that risk for TD may be associated to immune processes allied to the progression of fibrosis in patients with HCV. In our opinion, this study is of clinical interest and the first to study TD among different stages of fibrosis in HCV. Patients with more advanced chronic hepatitis C require closer follow up to diagnose and manage TD, including a high level of suspicion for thyroid carcinoma. This is particularly important in sub populations with more rapid liver disease progression as Latinos, HCV/HIV co-infected patients and HCV patients with concomitant alcohol use.

More research to study the immune and genetic aspects of liver disease progression and the autoimmune manifestations of the disease, including TD are needed to optimize diagnosis and management.

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