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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Prevalence of thyroid disorders and the correlation of thyroid profile with liver enzymes, serum activin-A and follistatin during the treatment of patients with chronic hepatitis C genotype 1 and 4

Kronik hepatit C genotip 1 ve 4 hastaların tedavisi sırasında tiroid bozuklukları prevalansı ve tiroid profili ile karaciğer enzimleri, serum activin-A ve follistatin arasındaki ilişki

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ABSTRACT

Objectives Chronic hepatitis C (CHC) and peg-interferon-α (Peg-INF-α) modulate serum activins and follistatin and are associated with thyroid disorders (TD).The aim of this study was to determine the frequency of CHC induced TD and to investigate the correlation of liver damage, serum activin-A and follistatin with the thyroid function parameters and thyroid autoantibodies.

Methods: The study was cross-sectional and sera were obtained from 132 patients with CHC who were divided into 3 groups: 56 patients with no treatment, 30 after 24 weeks of Peg-INF-α and 46 at the end of 48 weeks Peg-INF-α. Thyroid stimulating hormone (TSH), free thyroxin (FT4), thyroid antibodies (Tabs), serum activin-A and follistatin levels were measured using ELISA.

Results: Thyroid disorders were detected in 15% (n=20), more frequent in females (70%) and the majority were autoimmune thyroiditis (80%). TSH receptor antibodies (TSHR-Abs) were significantly prevalent compared to the other antibodies ($p<0.05$) and significantly increased in the 24 weeks group ($p<0.05$). TSH and FT4 correlated significantly with liver enzymes ($p<0.05$). There was no significant difference in activin-A and follistatin values between thyroid disorder and euthyroids. However, significant correlations were found between TSHR-Abs concentration with follistatin, activin-A and activin/follistatin ratio ($p<0.05$).

Conclusion: Thyroid disorders induced by CHC and/or Peg-INF-α were common in our patients, more prevalent in females and the majority are autoimmune. Additionally, activin-A and/or follistatin could be involved in the induction/aggravation of TSHR-Abs. Further studies are needed to confirm our findings and to explore the mechanisms by which CHC induces thyroid disorders. *J Clin Exp Invest 2014; 5 (3): 343-353*

Key words: Chronic hepatitis C, thyroid disorder, activin-A, follistatin, Saudi Arabia

ÖZET

Amaç: Kronik hepatit C (KHC) ve Peg-interferon- alfa (Peg-INF-α) serum aktivinleri ve follistatini düzenler ve tiroid hastalıkları (TH) ile birliktedir. Bu çalışmanın amacı KHC'nin indüklediği TH sıklığını belirlemek ve karaciğer hasarı, serum activin-A ve follistatin'in tiroid fonksiyonları ve tiroid otoantikorları ile ilişkisini araştırmaktır.

Yöntemler: Bu çalışma kesitsel olup, 3 gruba ayrılan 132 KHC'li hastadan serumları alınmıştır: Tedavisiz 56 hasta, 24 haftalık Peg-INF-α tedavisi almış 30 hasta ve Peg-INF-α tedavisi tamamlanmış 46 hasta. Tiroid stimülan hormon (TSH), serbest tiroksin (FT4) ve tiroid otoantikorları, serum activin-A ve follistatin düzeyleri ELISA yöntemiyle ölçüldü.

Bulgular: Hastaların %15'inde (n=20) tiroid hastalığı saptandı, bunların çoğunluğu (%80) otoimmün tiroidit olup, kadınlarda daha sık (%70) idi. TSH reseptör antikorları (TSHR-Abs), diğer antikorlardan anlamlı yüksek bulundu ($p<0,05$) ve 24 haftalık tedavi grubunda anlamlı artmıştı ($p<0,05$). TSH ve FT4 karaciğer enzimleri ile anlamlı körele idi ($p<0,05$). Tiroid hastalığı olanlarla ötiroid olanlar arasında activin-A ve follistatin değerleri bakımından anlamlı fark yoktu ($p>0,05$). Ancak TSHR-Abs ile follistatin, activin-A ve activi/follistatin oranı arasında anlamlı korelasyonlar saptandı ($p<0,05$) .

Sonuç: Hastalarımızda KHC ve/veya Peg-INF-α'nın indüklediği tiroid bozuklukları yaygın olup, bunların çoğu otoimmundur ve kadınlarda daha sık idi. İlaveten, activin-A ve/veya follistatin TSHR-Abs oluşumu/agrevasyonunda etkili olabilir. Sonuçlarımızı doğrulamak ve KHC'daki tiroid hastalıkları oluşum mekanizmalarını açıklamak için ilave çalışmalara gereksinim vardır.

Anahtar kelimeler: Kronik hepatit C, tiroid hastalığı, activin-A, follistatin, Suudi Arabistan

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INTRODUCTION

The treatment of chronic hepatitis C infection (CHC) is currently based on a combination of pegylated interferon-α (Peg-INF- α) and ribavirin and the treatment duration depends on the viral genotype [1]. One of the most prevalent CHC and Peg-INF-α extrahepatic disorders is thyroid dysfunction [2]. Types of thyroid disorders (TD) associated with HCV and Peg-INF-α based therapy range from subclinical to overt hypo- and hyperthyroidism due to autoimmune and non-autoimmune predisposition [3]. The prevalence of HCV and Peg-INF-α induced thyroiditis ranges between 2.5% to 35% [4,5].

The most common form of thyroid autoimmunity during CHC is the presence of thyroid antibodies (Tabs) without clinical disease [6]. Antibodies include thyroid peroxidase antibodies (TPO-Abs), TSH receptor antibodies (TSHR-Abs) and thyroglobulin antibodies (TG-Abs); and patients may be positive for one or more of these antibodies [3,7,8]. However, about 50% of patients who develop thyroid dysfunction during Peg-IFN-α therapy do not develop Tabs, suggesting that thyroid dysfunction could also be mediated by a direct effect on thyroid cell function rather than immune mediated mechanism [9]. IFN induced non-autoimmune thyroiditis exists in two forms, destructive thyroiditis and hypothyroidism [3].

Activins are cytokines that belong to the transforming growth factor-β superfamily and follistatin is the main regulator of the local bioactivity of activins since binding of activins to follistatin is almost irreversible [10]. Activins are involved in the pathogenesis of inflammatory and autoimmune human diseases [11,12]. Activin-A can have both pro- and anti-inflammatory actions depending on both cellular and temporal contexts [12].

Activins are secreted in the cytoplasm of the thyroid follicle cells [13,14], indicating an active synthesis and it has been suggested that they are involved in the stimulation of DNA synthesis and proliferation of thyrocytes of porcine thyroid cells in vivo [15]. The expression of activins' intracellular mediators (Smad) have also been reported in thyroid cells and in the nucleus of thyroid tumour cells, indicating propagation of activin signalling in thyrocytes [16].

Additionally, a slight increase in activin-A was associated with hyperthyroidism [17]. While a significant increase of activin-A was associated with hypothyroidism [18]. The evidence that immunostaining for activin A in thyroid follicular cells is more intense in patients with Graves' disease than in normal subjects [19]. Serum levels of activin-A are higher in goitrous patients with autoimmune thyroiditis more than control subjects [18].

Currently, there is no report from Saudi Arabia or other Arabian Gulf countries about the prevalence of thyroiditis in CHC patients with or without treatment with Peg-INF-α based therapy. Additionally, we have previously reported that CHC and Peg-INF-α based therapy modulate the serum concentrations of activin-A and follistatin [20,21]. The present study was therefore conducted to measure the frequency and types of thyroid disorders associated with CHC and/or its therapy in treatment naïve Saudi patients diagnosed with HCV genotype 1 and 4. We also measured the correlation between liver damage, serum activin-A, follistatin and activin/follistatin ratio with the thyroid function parameters at the time of diagnosis, after 24 weeks of treatment initiation and at the end of the treatment protocol of 48 weeks.

METHODS

Ethical approval

The study was approved by the institutional review board and ethics committee of King Abdullah Medical City (IRB 12-028). All samples were collected following obtaining informed written consent from all the participants.

Study design

This was a cross-sectional study. Serum samples were collected from 132 Saudi patients diagnosed with CHC genotype 1 or 4 and for whom positive polymerase chain reaction following reverse transcription was positive for HCV RNA according to the principle inclusion and exclusion criteria (table 1). The patients were divided according to the timing of the samples into 3 groups:

A. No treatment group (NT): This group included 56 patients according to the inclusion and exclusion criteria and who did not start their treatment protocol. The patients consisted of 24 males and 32 females with age range (22-70 years). Thirty two cases were positive for viral genotype 4 and 24 for genotype 1.

B. 24 weeks group (24W): This group consisted of samples collected from 30 patients who had Peg-INF-α based therapy for 24 weeks. The group included 20 males and 10 females with age range of (21-64 years). Sixteen cases were genotype 4 and 14 genotype 1.

Table 1. Principles inclusion and exclusion criteria

Principal inclusion criteria	Principle exclusion criteria
Patient age ≥ 18 years.	Patient age < 18 years.
HCV RNA positive	Previous non-responders/relapse
No concurrent infection with HBV or HIV	Solid organ transplant (renal, heart, or liver, etc.)
Dual therapy using peg-INF-α 2a or 2b with ribavirin	Mono- or triple based therapy
Treatment naïve patients	Autoimmune condition (type 1 DM, rheumatoid arthritis, etc.)
Compensated liver disease (e.g. no liver cirrhosis, failure or cancer) & APRI ≤ 1.2	History or current thyroid disease
Acceptable hematological and biochemical indices	Uncontrolled type 2 DM and HTN
No or controlled type 2 DM and hypertension	Concurrent chronic disease (Renal failure, CHD, etc.)

DM: Diabetes mellitus, HTN: Hypertension, CHD: Coronary heart disease

C. End of treatment group (ET): The final group included samples collected from 46 patients at the end of a 48 weeks treatment protocol. The patients were 24 males and 22 females with age range (19-61 years). Genotype 4 and 1 were positive in 28 and 18 cases, respectively.

All treated patients received PEG-INF-α-2a (Pegasys, Roche, Basel, Switzerland) at a dosage of 180 µg per week in combination with daily dose of ribavirin (Copegus, Roche) based on the body weight (1000 mg if < 75 kg or 1200 mg if ≥ 75 kg).

The results of liver function parameters and viral load at the time of sample collection were performed as part of the routine laboratory follow-up.

The study measured the prevalence of thyroid disorders and the different types of thyroid antibodies in all groups. We also measured concentrations of activin-A and follistatin in all samples and positive samples for the different thyroid antibodies were compared to the negative samples within the same group. Furthermore, serum TSH and free T4 were measured in all samples and correlation studies were conducted with the different thyroid antibodies, liver enzymes, activin-A and follistatin.

Calculation of APRI

APRI was calculated using the following equation: (AST/upper limit of normal)/platelet count (X 109/L) X 100. The interpretation of the APRI results was performed according to previously published studies [20,21]. All samples were collected following the calculation of their APRI and only samples with ≤ 1.2 were included into the study to avoid samples collected from patients suffering from liver cirrhosis.

Measurement of TSH and free T4

The quantitative measurement of thyroid stimulating hormone (TSH) and free thyroxin hormone (FT4) was done using electro-chemiluminescence immunoassay (ECLIA) on Cobas e411 (Roche Diagnostics International Ltd, Switzerland) according to the manufacturer protocol. The reference range according to the manufacturer for TSH and free T4 was 0.27−4.20 µIU/mL and 12-22 pmol/L, respectively. The detection sensitivity was 0.005 µIU/mL for TSH and 0.3 pmol/L for FT4. The intra- and interassay coefficients of variations for FT4 were 1.7 and 3.9% and for TSH were 1.4% and 3.4%, respectively. The diagnosis and classification of thyroid abnormalities were according to the guidelines of the National Academy of Clinical Biochemistry (NACB) for laboratory diagnosis and monitoring of thyroid diseases [22]. Hypothyroidism was considered when TSH > 4.5 µIU/mL and the level of FT4 defined the type as either primary (FT4 < 12 pmol/L) or subclinical (FT4 ≥ 12 pmol/L). Hyperthyroidism was considered for TSH < 0.10 µIU/mL and FT4 > 22 pmol/L.

Measurement of thyroid autoantibodies

ELISA was used to detect the IgG antibodies against TSH receptor (TSHR-abs), TPO (TOP-abs) and TG (TG-abs) (Human Diagnostica, Germany) by following the manufacturer's instructions and the optical density of the plates was measured within 10 min using a plate reader at 450 nm. The cut-off values according to the manufacturer's instructions for a positive test for TSHR-abs was > 1.5 IU/l, negative was < 1 IU/l and equivocal was between 1.1-1.5 IU/l. Results for TPO antibodies were considered negative if < 80 WHO-IU/ml, equivocal between 80-

150 WHO-IU/ml and positive if > 150 WHO-IU/ml. For TG antibodies, results were considered negative if < 80 WHO-IU/ml, equivocal if between 80-200 WHO-IU/ml and positive if > 200 WHO-IU/ml.

The IMTEC-TSH Receptor-Antibodies ELISA has a sensitivity of 100% and a specificity > 95%. The kit has a lower detection limit of 0.21 IU/L and the inter-assay and intra-assay imprecision is CV 10.9 – 12.9 % and CV 2.2 – 7.1 %, respectively. The IMTEC-TPO-Antibodies has a sensitivity of 92.9% and a specificity of 97.6%, and the analytical sensitivity of the kit is ≤ 3.0 WHO-IU/ml. The inter-assay and intra-assay imprecision is CV 11.1% and CV 8.9%, respectively. The IMTEC-TG-Antibodies ELISA kit has a sensitivity of 92.8% and a specificity of 97.9%, and the analytical sensitivity of the kit is ≤ 6.0 WHO-IU/ml. The inter-assay and intra-assay imprecision is CV 8.2% and CV 8.3%, respectively.

Measurement of human activin-A and follistatin serum concentrations

ELISA was used for quantitative measurement of human activin-A and follistatin in all samples (R&D systems, USA) as previously described. As reported by the manufacturer, the lowest detection limit of activin-A by the used kit is 3.7 pg/mL and the upper limit is 1500 pg/mL. The intra-assay and inter-assay precisions of the kit are 4.3% and 5.8%, respectively. The kit cross reacts by 0.2% and 0.45% with inhibin-A and activin-AB, respectively. The detection range of the follistatin kit is 250-16000 pg/mL and the minimum detectable dose is 83 pg/mL. The intra-assay and inter-assay precisions are <3% and <9%, respectively. Activin-A/follistatin ratio index (AFRI) was calculated as follow: [Activin-A/follistatin x 100].

Statistical analysis

Statistical analysis of the results was performed using SPSS version 20. The Chi square (X2) test was used for frequency analysis. According to data normality, either student's T test or Mann-Whitney U test was used to compare between 2 groups. Furthermore, one way ANOVA followed by Tukey post hoc test or Kruskal–Wallis followed by Dunn's post hoc test were used to compare between more than 2 groups depending on the data homogeneity. Correlations were determined using Pearson's test. P value < 0.05 was considered significant.

RESULTS

Demographic results and general laboratory parameters

There was no significant difference in the mean age, the distribution of gender, viral genotype and viral load at diagnosis, liver enzymes and thyroid hormones either between the three study groups or within each group (table 2).

Table 2. Demographic and laboratory characteristics of all study participants according to the different study groups

		No treatment (n = 56)	24 weeks (n = 30)	End of treatment (n = 46)
Age (years)		50.1 ± 16.3	46.4 ±13.3	44.1 ± 14.6
Gender	Male	46.4%	66.7%	39%
	Female	53.6%	33.3%	61%
Genotype	G1	43%	46.7%	50%
	G4	57%	53.3%	50%
Viral Load at diagnosis (IU/mL)		1125694 ± 555544	999187 ± 400348	1048288 ± 303944
Albumin (g/dL)		3.8 ± 0.47	4.06 ± 0.22	4.06 ± 0.42
ALP (IU/L)		116 ± 55.6	79.4 ± 39.1	93.7 ± 51.6
ALT (IU/L)		45.5 ± 19.9	43.2 ± 25.6	34.8 ± 23.7
AST (IU/L)		47 ± 18.6	38.8 ± 17.8	33.4 ± 15.4
APRI		0.68 ± 0.37	0.59 ± 0.31	0.64 ± 0.35
TSH (μIU/L)		2.4 ± 1.1	2.7 ± 1.5	3 ± 2.1
FT4 (pmol/L)		14.2 ± 3.3	14 ± 3.8	13.4 ± 3.8

Prevalence and types of thyroiditis in the different study groups

Thyroid disorder was detected in 20 patients (15%) of the study participants. Six cases were found in the 'No treatment' group, 8 patients in the '24W group' and 6 in the 'End of treatment' group. Regarding gender, 70% of thyroiditis was observed in females (n = 14) and 30% in males (n = 6) with female: male ratio 2.3:1. Half of the patients (n = 10, 50%) had subclinical thyroiditis and the remainder had clinical thyroiditis. The prevalence of hypothyroidism and hyperthyroidism was 60% vs. 40%, respectively (Figure 1). Sixteen patients had autoimmune thyroiditis (80%) and four had non-autoimmune thyroid disease (20%) (Figure 2).

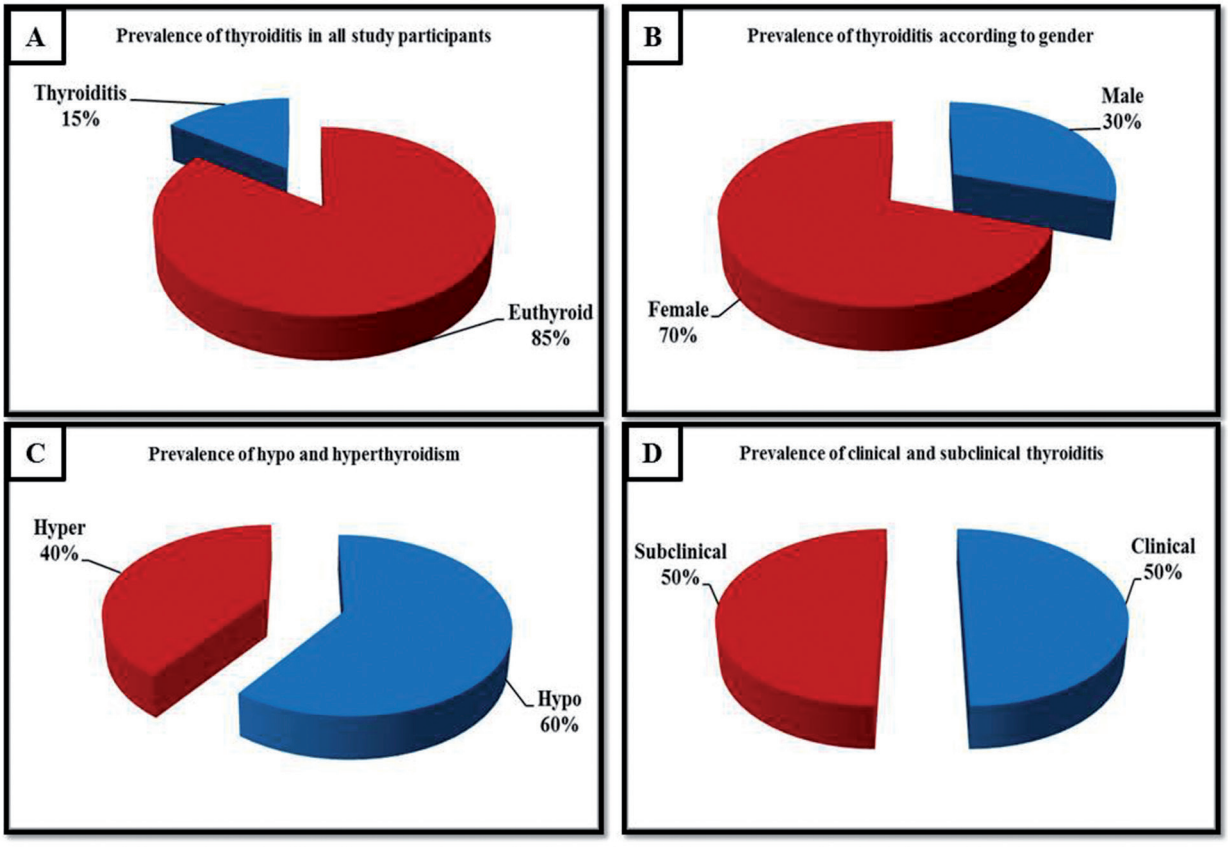

Figure 1. The prevalence of thyroiditis (A) in all study participants, (B) in male and female participants (C) according to hypo- or hyperthyroidism and (D) according to clinical and subclinical presentation

Prevalence of thyroid antibodies in the different study groups

The three thyroid antibodies were detected in all groups except for the 'No treatment group' in which all cases were negative for TPO antibodies (Figure 3). The total number of positive cases in the 3 groups for TSHR-Abs (n = 64, 48.4%) was significantly higher when compared with the other 2 antibodies (P = 0.1X10-4) (Figure 3). Furthermore, there was no significant difference (P = 0.4) between the number of positive cases for antibodies against TPO (n = 6, 4.5%) when compared to TG (n = 16, 12.1%).

TSHR-Abs were detected in the 'NT' (n = 20, 35.7%), '24W' (n = 22, 73.3%) and 'ET' (n =22, 47.8%) groups. There was a significant increase in the prevalence of TSHR-Abs following treatment with Peg-INF-α for 24 weeks (P = 0.02) followed by a non-significant decline at the end of the 48 weeks treatment protocol. However, there was no significant difference in the number of positive cases between the 'NT' vs. 'ET' groups and the '24W' vs. 'ET' groups (P > 0.05) (Figure 3).

Serum concentrations of TSHR-Abs showed a similar pattern to the prevalence as a significant increase was detected between the '24W' (4.25 ± 1.08 IU/mL) and 'NT' groups (2.1 ± 0.36 IU/mL) (P = 0.0004). Additionally, a significant difference was observed between the '24W' and 'ET' (2.3 ± 0.49 IU/

mL) groups (P =0.001). There was no significant difference between the 'NT' and 'ET' groups (P = 0.4) (Figure 3). Furthermore, a significant increase in the concentration of TSHR-Abs was observed in the autoimmune thyroiditis (3.88 ± 0.43 IU/mL) compared to the positive cases for TSHR-Abs in the euthyroid group (2.1 ± 0.25 IU/mL) (P = 0.003) (Figure 3).

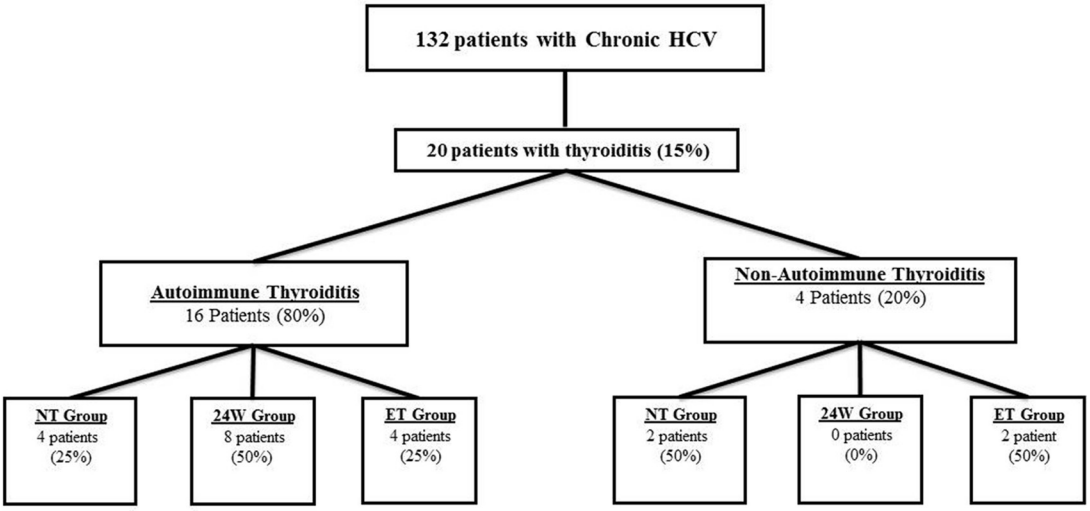

Figure 2. The prevalence of thyroiditis in the 132 study participants and the prevalence in the 'No treatment' group (NT), '24 weeks' group (24W) and the 'End of Treatment' group (ET) according to auto- and non-autoimmune

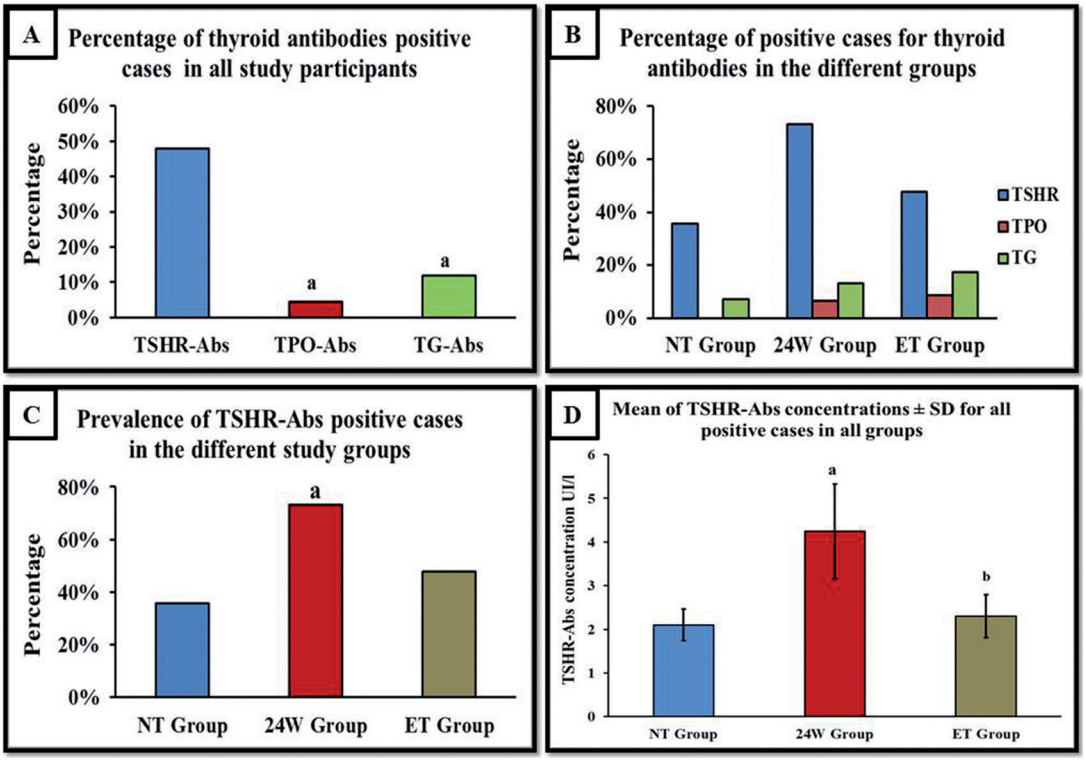

Figure 3. Prevalence of (A) thyroid autoantibodies in all study participants, (B) different thyroid autoantibodies according to study groups, (C) TSHR-Abs according to study groups (a = $p < 0.05$ compared to NT group) and (D) mean ± SD of serum TSHR-Abs concentrations in the positive cases in each study group (a = $p < 0.05$ compared to NT group and b = $p < 0.05$ compared to 24W group).

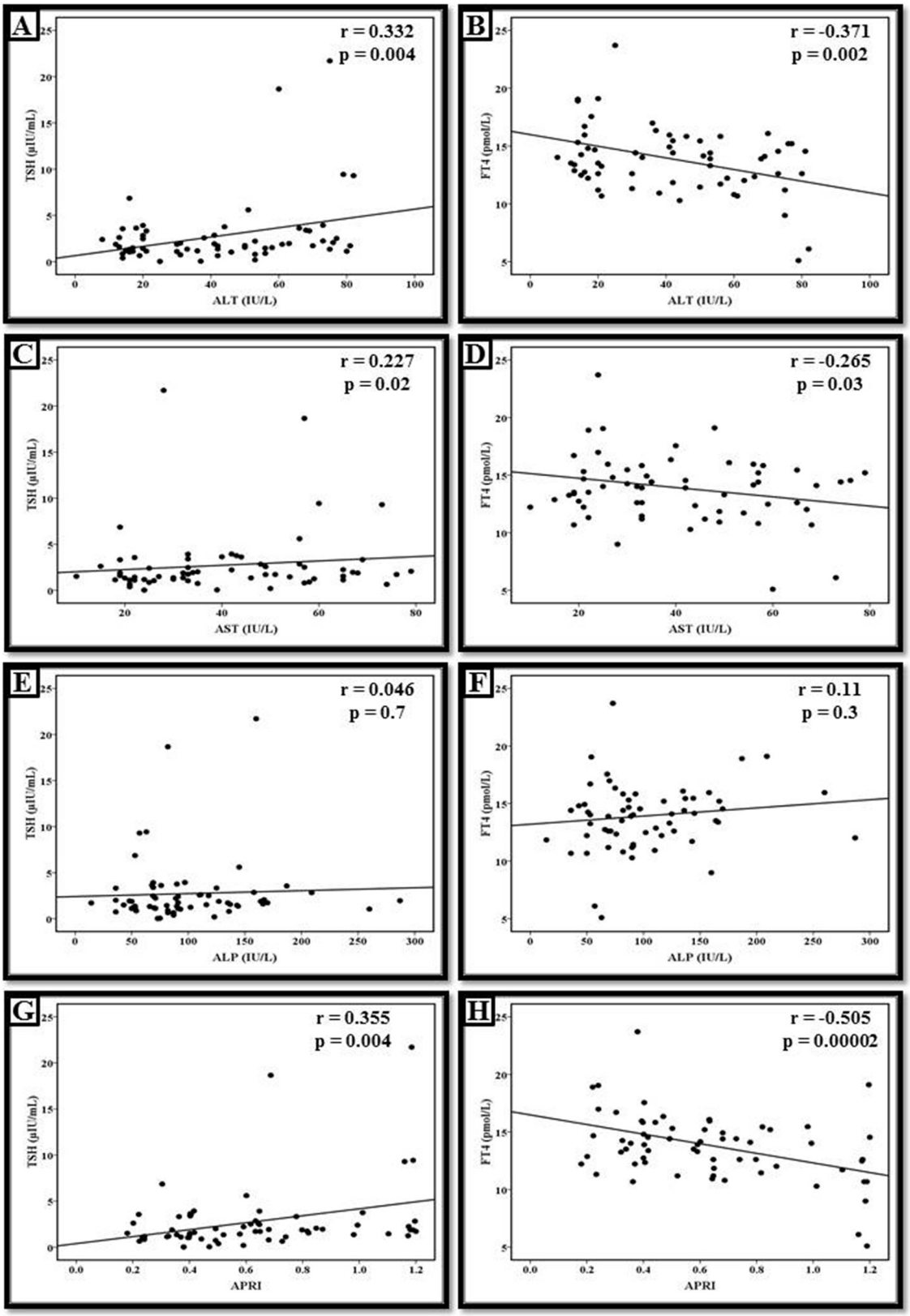

Figure 4. Correlation of ALT (A & B), AST (C & D), ALP (E & F) and ASPRI (G & H) with TSH (left column) and free T4 (Right column)

Correlation between liver enzymes and thyroid hormones

Liver enzymes and APRI correlated significantly with TSH and free T4 (Figure 4). ALT significantly correlated with TSH (r = 0.332, P = 0.004) and inversely with free T4 (r = -0.371, P = 0.002). Additionally, a similar significant pattern of correlation, was observed for AST with TSH (r = 0.277, P = 0.02) and free T4 (r = -0.265, P = 0.03). The strongest significant correlation with thyroid hormones was observed with APRI (r = 0.355, P = 0.004; r = -0.505, P = 0.00002) for TSH and free T4, respectively. ALP did not correlate with neither TSH (r = 0.046, P = 0.7) nor free T4 (r = 0.11, P = 0.3) (Figure 4).

Activin-A and follistatin in hepatitis C induced thyroiditis

There was no significant difference in serum activin-A, follistatin and activin/follistatin ratio (A/F) between autoimmune thyroiditis and euthyroid cases (Table 3) and between positive and negative cases for TSHR-Abs within each study groups (P > 0.05) (Table 4).

There was a significant negative correlation between serum activin-A and TSHR-Abs concentration (r = -0.206, P = 0.01). Furthermore, a significant positive correlation for TSHR-Abs concentration with follistatin (r = 0.303, P = 0.0001) and a significant inverted correlation with A/F ratio (r = -0.342, P = 0.0005) was observed (Table 5).

Table 3. Mean of activin-A, follistatin and AFRI ratio ± SD in autoimmune thyroiditis (AT) and euthyroidism (EU) within each of the study group. No significant difference was observed between positive and negative cases within each group.

	NT group		24W group		ET group	
	EU	AT	EU	AT	EU	AT
Activin-A (pg/mL)	864.2 ± 233	822.8 ± 163	424 ± 193	382.7 ± 107	471.7 ± 127.1	695.5 ± 227.1
Follistatin (pg/mL)	473.9 ± 201.5	408.6 ± 265.6	807.8 ± 285	644 ± 105.9	684.9 ± 362.2	425.8 ± 187.1
AFRI	195 ± 71	248 ± 87	77 ± 27	61 ± 22	118 ± 31	170 ± 74

Table 4. Mean of activin-A, follistatin and AFRI ± SD in positive (+) and negative (-) cases of TSHR-Abs within each of the study group. No significant difference was observed between positive and negative cases within each group.

	NT group		6MT group		ET Group	
	- TSHR-Abs	+ TSHR-Abs	- TSHR-Abs	+ TSHR-Abs	- TSHR-Abs	+ TSHR-Abs
Activin-A (pg/mL)	830.8 ± 348.6	929.1 ± 248.8	508 ± 232	479 ± 174.7	540.5 ± 189.3	478 ± 120
Follistatin (pg/mL)	451.4 ± 193.6	507.6 ± 202.8	1076.8 ± 382	850.5 ± 277.6	674.8 ± 211.5	761.2 ± 227.2
AFRI	194 ± 68	207 ± 81	59 ± 30	78 ± 32	149 ± 81	105 ± 42

Table 5. Results of correlation analysis using Pearson's test for serum activin-A, follistatin and AFRI with TSH, FT4, TSHR-Abs, TPO-Abs and TG-Abs

		TSH	FT4	TSHR-Abs	TPO-Abs	TG-Abs
Activin-A	**r**	0.023	-0.064	-0.206*	0.027	0.089
	p	0.7	0.4	0.01	0.7	0.3
Follistatin	**r**	-0.091	-0.053	0.304*	-0.086	0.005
	p	0.7	0.5	0.0002	0.3	0.9
AFRI	**r**	0.019	0.062	-0.342*	0.079	0.007
	p	0.8	0.4	0.00001	0.3	0.9

DISCUSSION

The current research is the first to report the prevalence and types of thyroid disorders associated with CHC infection with or without Peg-INF-α therapy in treatment naïve Saudi patients. Furthermore, this is the first study to report significant correlations between serum concentrations of TSH receptor antibodies with serum Follistatin, activin-A and activin-A/follistatin ratio. However, there was no significant difference in serum concentrations of activin-A and follistatin between positive and negative cases for TSH receptor antibodies. Our results suggest that activin-A and/or follistatin may play a role in the development of TSH receptor antibodies. Finally, significant correlations between ALT, AST and APRI were also observed with the levels of serum TSH and T4, suggesting that liver damage following CHC is associated with hypothyroidism.

There are several drawbacks in our study. A limitation of our study is that it presents data from a small sample size and increasing the number of patients would increase the statistical power of the study to reflect the actual prevalence of CHC and Peg-INF-α induced thyroid diseases in Saudi patients. However, we were able to detect thyroid disorders in 15% and TSH receptors antibodies in 48.4% of the study population, suggesting that thyroid disorders are a common complication of CHC and its currently used treatment in Saudi patients.

A further limitation is that we did not measure free T3, total T3 and total T4 to appropriately evaluate the thyroid functions. Nevertheless, the routine screening and clinical biochemical diagnosis of thyroid disorders are essentially based on measuring TSH and free T4. Another limitation is that we adopted a cross-sectional design and performing a longitudinal prospective study would reveal the accurate timing of thyroiditis development and would allow proper follow-up for patients to determine the prognosis and rate of recovery.

The reported prevalence of HCV and Peg-INF-α induced thyroiditis in developed countries ranges between 2.5% to 35% [23]. Currently there are only two available reports from the Middle East and the prevalence ranged between 8.5 and 20% [24,25]. Females are at higher risk of developing thyroiditis following CHC and peg-INF-α [3]. Furthermore, hypothyroidism has also been reported to be more common than hyperthyroidism [26,27]. Similar results were also reported form the UK[28], Taiwan [29] and Australia [30]. Our study supports the previous findings as it showed an overall 15% prevalence of CHC and peg-INF-α induced thyroid disorders in Saudi patients, the majority were females and 60% of the patients had hypothyroidism.

Thyroid autoimmunity are significantly more common with CHC, which is associated with the development of the different types of thyroid autoantibodies (Tabs) [31]. Patients infected with HCV present 40-42% of detectable Tabs levels; whereas in patients with hepatitis B virus, the index varies from 5 to 10%[31]. Our results correlate with the previous studies as we have detected Tabs in 60% of our patients. However, the higher prevalence in our study could be due to differences in the study design and the sample size, which is smaller in our study, or different environmental and genetic predispositions [27].

The most commonly studied and associated Tabs with HCV and peg-INF-α induced thyroiditis in the literature are TPO and TG antibodies [3]. However, patients with destructive thyroiditis were also reported to have inhibitory TSHR-Abs[32]. The most prevalent Tabs in our study were TSHR-Abs and the lowest were TPO-Abs. Additionally, TPO-Abs were only detected during the course of therapy and at the end of treatment, suggesting a role for INF-α based therapy in their synthesis.

The differences between our findings and the previous reports could be due to the non-longitudinal design of our study and the small sample size, which may have underestimated the prevalence of TPO and TG antibodies. This variability can also be attributed to the diverse genetic predisposition of the subjects [27,33]. However, the detected significant difference in the prevalence of TSHR-Abs in our study suggests that the development of antibodies against TSH receptor are common in Saudi patients during CHC and during the treatment with Peg-INF-α based therapy.

Strong correlations between liver damage and thyroid disorders have been reported [34]. Non-alcoholic fatty liver diseases and abnormal liver enzymes are significantly associated with hypothyroidism and the the prevalence of liver diseases and enzymes increase steadily with increasing grades of hypothyroidism [34]. Furthermore, a decrease in serum T3 concentration and T3:T4 ratio is frequently observed in patients with liver cirrhosis probably due to impaired conversion of T4 to T3 in the liver [35]. Thyrotoxicosis is also associated with a variety of abnormalities of liver function [36] and results from a recent study suggests that low FT4 concentrations are associated with hepatic steatosis

[37]. Serum TSH level was also significantly higher in nonalcoholic fatty liver diseases and it has also been suggested that measurement of FT3 and FT4 levels may all be useful as predictors of mortality in intensive care patients who have cirrhosis [38]. Our findigns are in agreement with the previous studies as they showed significant correlations between AST and ALT with both TSH and FT4 in patients with CHC and the strongest correlation was observed for thyroid hormones with APRI.

APRI is used as a predictor of liver fibrosis and cirrhosis in CHC as a replacement of liver biopsy in a substantial proportion of patients [39]. Several studies have confirmed the significant correlation between APRI and both the stage of liver fibrosis and the grade of activity [21]. Therefore, we suggest that the degree of liver damaged associated with CHC could be responsible for the non autoimmune thyroid abnormalities. However, investigating the prevalence of thyroid disorders associated with different stages of liver fibrosis and cirrhosis due to CHC is required to support our hypothesis.

Harada et al. confirmed that pathological expression of activin-A was associated with hyperthyroidism [17], while a significant increase of activin-A was associated with hypothyroidism and serum levels of activin-A were higher in goitrous patients with autoimmune thyroiditis[18]. The present work disagrees with the previous observations as it showed no significant difference in the concentrations of activin-A between autoimmune thyroiditis and euthyroid cases within each study groups. Similar results were also observed for follistatin and activin/follistatin ratio (A/F). The non-significant difference could be due the small sample size included in the study. However, the current study is the first to report that serum concentration of TSHR-Abs signifantly correlates positively with serum follistatin and negatively with activin-A and A/F ratio.

Activin-A has been established as a main regulator of immune responses [20,21] and pathological activin-A levels have been associated several autoimmune diseases such as rheumatic diseases [40], systemic lupus erythematosus [41] and allergic airway diseases [42]. Therefore, we suggest that activin-A and/or follistatin are involved in HCV and Peg-INF-α induced autoimmune thyroiditis.

In conclusion, thyroid disorders induced by HCV and peg-INF-α treatment are common in Saudi patients, with higher prevalence in females and the majority is autoimmune in nature. Additionally, activin-A and/or follistatin could be involved in the induction/agrevation of TSH receptors antibodies. Further studies are needed to confirm the detected prevalence of thyroid disorders in our study and to explore the role of activins and their binding protein in hepatitis C induced thyroiditis.

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