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Thyroid Peroxidase Antibodies and Thyroid Dysfunction in Patients with Chronic Hepatitis C Treated with Conventional Interferon and Ribavirin

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ABSTRACT

***Objective*:** To determine the frequency of thyroid dysfunction among patients with chronic hepatitis C receiving Conventional Interferon and Ribavirin combination therapy and to compare the frequency of thyroid dysfunction among patients with thyroid peroxidase positive and negative antibodies among these patients.

***Study Design*:** Cross-sectional study.

***Place and Duration of Study*:** Gastroenterology Department of Medicine, Combined Military Hospital, Lahore Pakistan, from Jan to Jul 2017.

***Methodology*:** Patients reporting chronic hepatitis C, fulfilling the required selection criteria, and received conventional Interferon and Ribavirin therapy were included. A thyroid Function test was performed at enrollment, week-12 and week- 24. The outcome was measured at week 24 by the end of treatment. Anti-Thyroid Peroxidase antibody level was measured at the time of enrollment.

***Results*:** One hundred and fifty-seven patients were enrolled in the study. There was no difference in demographic data comparison. At the end of therapy, 5 (29.41%) male patients and 12 (70.59%) female patients developed TD, with the *p*-value of 0.019, which is statistically significant. 28 (17.83%) out of 157 patients had positive anti-TPO at baseline. The inferential analysis showed that there were significantly higher positive rates of pre-treatment anti-TPO in patients with TD (16 (94.11%) out of 17) when compared with patients with normal baseline anti-TPO (1 (5.89%) of 17), with the *p*-value of 0.001.

***Conclusion*:** Pre-treatment anti-Thyroid Peroxidase antibodies and female gender are the most significant risk factors for developing Thyroid Dysfunction during Interferon and Ribavirin therapy.

Keywords: Chronic hepatitis C, Interferon alpha, Interferon-induced thyroid disorders, Thyroid disorder.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a global problem. Worldwide there are around 130-170 million chronic carriers,[1] which is approximately 3% of the world population, per the World Health Organization (WHO). Therefore, HCV infection is one of the major epidemics, infecting young adults in both developed and developing countries. Patients infected with HCV are at risk of developing liver cirrhosis and hepatocellular carcinoma, which, in its natural, unfavourable course, is a cause of morbidity and mortality.[1] The estimated liver-related mortality is 350,000 people/ year.

HCV affects not only the liver but also various extrahepatic tissues and organs; hence the concept of systemic HCV infection has emerged.[2] Various mechanisms have been proposed for the extrahepatic manifestations, including immunological reaction, virus invasion and replication in the affected extrahepatic organs.[2,3] Conversely, interferon-alpha is a powerful inducer of autoimmunity. It acts as an additional risk factor for the autoimmune process.[4,5]

HCV causes frequent and polymorphous extrahepatic manifestations.[6,7] It is estimated that around two-thirds of patients with HCV infection develop extrahepatic manifestations. Chronic HCV has a higher mortality rate for extrahepatic complications.

HCV infection may lead to hypothyroidism, hyperthyroidism, or biphasic thyroiditis.[2,6] The most common thyroid disorder observed is Hashimoto's thyroiditis. There is a wide variety of anti-thyroid antibodies (anti-thyroid peroxidase antibody (anti-TPO Ab), anti-thyroglobulin antibody, and anti-thyroid microsomal antibody) varying in prevalence from 2% to 48% in HCV patients treated with interferon-alpha. Anti-thyroid antibodies are also present in the serum of HCV-infected patients before IFN α treatment.

This study was conducted in our setup to predict TD in our patients with conventional interferon

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therapy and to improve our monitoring while patients are on treatment. This study aims to add thyroid profile as part of a monitoring tool during conventional interferon therapy. In addition, with this study, we aim to add anti-TPO testing as part of the baseline before starting interferon therapy. This study will help improve the management protocol.

METHODOLOGY

The cross-sectional study was conducted at the Gastroenterology Department of Medicine, Combined Military Hospital, Lahore, from January to July 2017 after getting approval from Institutional Ethical Review Board (ERB Reference No. 204/2020). The Consecutive non-probability sampling technique was employed. A sample of 157 patients was estimated using WHO sample size calculator with confidence interval (CI) = 95%, anticipated population proportion = 23.4%1 and absolute precision=10%.

Inclusion Criteria: Patients of either gender between 18 to 50 years of age having chronic Hepatitis C with HCV RNA detected on PCR and treatment naïve status with either positive or negative baseline TPO antibodies were included in the study.

Exclusion Criteria: Patients with deranged thyroid profile, concomitant serious medical illnesses like malignancy, severe cardiopulmonary disease, decompensated Chronic Liver Disease, uncontrolled diabetes mellitus, co-infection with human immunodeficiency virus (HIV) or hepatitis B (HBV), goitre on clinical examination, peripheral blood leukocyte count < 3×10^9/L or platelet count < 70×10^9/L or haemoglobin level lower than 100 g/L, history of thyroid replacement therapy or anti-thyroid drugs, previous failure of response or relapses to Conventional Interferon therapy, pregnant and lactating women were excluded from the study.

Patients reported to CMH Lahore with Chronic HCV infection meeting the inclusion criteria were included in the study. Written informed consent was obtained from every patient included in the study.

After the initial evaluation, the blood sample of each participant was sent to the laboratory for baseline anti-TPO Ab level. According to the manufacturer's recommendations, the cut-off for anti-TPO antibodies was <20 AU/ml. Thyroid profile was taken at entry (0), week 12, and week 24 (outcome) of the treatment. The range for TSH was 0.4–4.5 mIU/ml, and for T4 was 8.0–21.0pmol/l. Reports verified by a Pathologist. Patients were given Conventional Interferon and Ribavirin therapy for 06 months. A persistent or intermittent elevation made the diagnosis of Chronic HCV of alanine aminotransferase (ALT, the upper limit of normal ALT is 40 IU/L) over six months, anti-HCV positivity and detection of HCV-RNA in the sera.

Additionally, genotyping and an Ultrasound abdomen were also done. The patient's baseline characteristics, routine laboratory data and virological information were recorded on predesigned proforma. All patients were treated with respect, and their comfort was cared for during the treatment. We strictly adhered to our exclusion and inclusion criteria to control confounders and bias in the study. The dosage of regular IFN-α ranged from 3 to 5 MU twice weekly, and Ribavirin was co-administered at a daily dose of 600 to 1200 mg, according to their body weight. The duration of follow-up was calculated as the time from the initiation of therapy until the last time the patient was seen at the outpatient clinic. Dose adjustments or therapy interruptions were made according to the specific characteristics of each patient and on specialists' recommendations.

Statistical Package for Social Sciences (SPSS) version 25.0 was used for the data analysis. Mean and standard deviation was calculated for the age, while percentage and frequency were computed for gender, anti-TPO Ab and TD. The Chi-square and Fischer exact tests were applied to see the statistical difference. The *p*-value of ≤0.05 was considered statistically significant.

RESULT

Our study included 157 individuals, among which 88 patients (56.1%) were males while 69 (43.9%) were females. The patients' age ranged from 18 to 50 years, with the mean age of 34.66±4.604.

Gender was associated with TD, be it hypothyroidism or hyperthyroidism, with the disease presenting in 5 (29.41%) males and 12 (70.59%) females. A significant association was found between gender and TD, with the *p*-value of 2.183, as shown in Table-I.

Table-I: Association of gender with thyroid dysfunction (n=157)

Gender	Thyroid Dysfunction n(%)		
	Yes n=17	No n=140	*p*-value
Male	5 (29.41)	83 (59.28)	2.183
Female	12 (70.59)	57 (40.72)	

Moreover, age was divided into two groups of 18-34 years and 35-50 years, with 9 (52.94%) and 8

(47.06%) individuals presenting with TD in each category, respectively. A significant association, as a result, was not observed between the age of the patient and TD (*p* value= 0.507), as shown in Table-II.

Table-II: Association of age with thyroid dysfunction(n=157)

Age Groups	Thyroid Dysfunction n(%)		
	Yes n=17	No n= 140	*p*-value
18-34 years	9 (52.94)	77 (55.00)	0.507
35-50 years	8 (47.06)	63 (45.00)	

Finally, a significant association was observed between anti-TPO Ab levels and TD. At baseline, 28 out of 157 patients had positive anti-TPO Ab. In the univariate analysis, there were significantly higher positive rates of pre-treatment anti-TPO Ab in patients with TD compared to patients with normal baseline anti-TPO levels, with the *p*-value of 0.001, as shown in Table-III.

Table-III: Association of anti-TPO Ab with thyroid dysfunction(n=157)

Anti-TPO Ab	Thyroid Dysfunction		
	Yes n (%)	No n (%)	*p*-value
Positive	16 (94.11)	12 (8.57)	0.001
Negative	1 (5.89)	128 (81.43)	

DISCUSSION

Thyroid disorders are common during interferon therapy. Obołończyk Ł revealed female gender has a higher incidence of developing TD with conventional interferon therapy than males.[1] There were a 5.76 times higher incidence of TD in women than men when the value of the remaining factors in the model was constant (p=0.024).[1] In the present study, positive anti-TPO titres ranging between 20-30 AU/ml were encountered in 13.3% of all patients, without any significant difference between males and females. However, high anti-TPO titres >30 AU/ml charac-teristic of autoimmune thyroiditis were significantly more frequent in females (5 females vs 2 males). These findings, taken together, suggest that female patients with HCV treated with conventional Interferon alpha and Ribavirin are at particularly high risk for developing TD.

A review article published in 2016 by Shen *et al.* revealed that the incidence of anti-TPO Ab was 1.96 fold higher in HCV-infected subjects than in controls.[2] TD has been observed with Peg Interferon as well. A study done by Yong Hwang revealed TD developed in 67 patients (27.7%) during the PEG-IFN/RBV treatment.[3] TD in HCV is one of the commonest extrahepatic manifestations observed by Vigano *et al.*[4] Fallahi *et al.*[5] and Di *et al.*[6] The incidence of TD in the present study is 10.8%. Our results are similar to the study done by Tran *et al.*[7] which showed the prevalence of TD before Interferon therapy ranges from 4.6 to 21.3%; during therapy, 1.1 to 21.3%; and after therapy, 6.7 to 21.3%.

Mammen *et al.*[8] showed similar results to our study. In their study, 20.8% of patients developed TD. The most common finding was low TSH, which occurred in 8.2% of patients, hypothyroidism in 6.1%, and biphasic thyroiditis in 6.6%. Multivariate logistic regression analysis showed that biphasic thyroiditis is associated with female gender and high pre-treatment TSH.[8] However, in the present study, we only studied the patients with normal Thyroid profiles, so that association with other factors like age, gender and pre-treatment anti-TPO Ab can be established. The present study also showed a positive association between the female gender and TD with Fischer exact value of 0.105 (standardized value 2.183). We have not studied the effect of smoking on the incidence of thyroiditis. However, Mammen *et al.* revealed in his study that smoking decreases the risk of thyroiditis.[8] Several studies have revealed that TD is a common autoimmune adverse effect of interferon therapy,[9] it is not a prognostic marker of liver fibrosis nor a predictor of therapeutic response. Thus further studies are needed to understand the pathogenesis of interferon-induced thyroid disease and the role of various viral, environmental and genetic factors in its aetiology and patient outcome. It will guide us in understanding the disease, improving patient care, earlier diagnosing TD, and a better therapeutic approach to interferon-induced TD. Another study explained that interferon-induced thyroiditis could be divided into two groups: autoimmune and non-autoimmune.[10] Autoimmune Thyroiditis can manifest as Hashimoto's thyroiditis or Grave's disease and occasionally may be associated with the production of thyroid autoantibodies (including Thyroid Peroxidase antibody) without clinical disease.[10] The strongest risk factors associated with an increased risk of developing thyroid dysfunction during Interferon alpha and Ribavirin therapy were pre-treatment TPO level and female gender. In our study, 70.5% of women and 29.4% of men developed Thyroid Dysfunction. Similarly, the present study showed that the presence of anti-TPO before treatment is a predictive factor of thyroid dysfunction. The development of thyroid dysfunction during Interferon Alpha and

Ribavirin combination viral therapy in 157 patients was 10.8%. Another study observed a similar relationship, showing that the positive percentage of TPO-Ab in the Thyroid Dysfunction group was significantly higher than that of patients without Thyroid Dysfunction (66.7 vs 16.7%, p=0.005).[11] A systemic review showed the overall frequency of newly developed thyroid dysfunction during IFN treatment was 12.8%,[12] which correlates with our results. A similar relationship has been observed between pre-treatment thyroid peroxidase antibody and thyroid dysfunction with Interferon therapy by Friedrich-Rust *et al*.[13]

The long-term outcome of these patients is still unknown.[14] In our study, thyroid dysfunction resolved spontaneously after completion of HCV therapy in 2 (11.7%) patients. However, thyroid disease may persist in some patients; thus, long-term treatment may be required. A study by Chang *et al*. showed the occurrence of Thyroid Dysfunction during the 13-year follow-up period; the patients treated with PEG-IFN were at a higher risk of suffering from Thyroid Dysfunction (p<0.001), according to the Kaplan-Meier analysis.[15] However, our study period was 6 months, and further study is needed to ascertain the long-term effects of Interferon on Thyroid Dysfunction.

In all these disorders, the presence of Thyroid autoantibodies prior to the initiation of Interferon therapy is an important risk factor for developing interferon-induced thyroiditis.[16,17,18] However, we did not check for the development of Thyroid autoantibodies in our patients because of cost-issue. We need better funding to establish this association in our population.

LIMITATIONS OF STUDY

Because of limited funds, we did not check the association between Interferon therapy and the development of anti-TPO Ab at the end of therapy.

CONCLUSION

Baseline anti-TPO Ab and female gender are independent risk factors for developing TD in patients with HCV treated with Conventional Interferon and Ribavirin.

Conflict of Interest: None.

Author's Contribution

AA: Concept and design, literature review, data collection and abstract, FM: Critical review, AA:, MAY Paper drafting, SKHSB: Literature review, data entry, proof reading, MUT: Data analysis,

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