

Original Research Article

Anti-thyroid antibodies in patients with HCV genotype 3a

Abstract

Background: Each year, 3 to 4 million people are infected with hepatitis C virus (HCV) infection and it is the major cause of liver disease worldwide. Patient of acute HCV infection is often asymptomatic but can present with fatigue and jaundice. About 80% of HCV infected individual's progress to chronic state. There is an increased prevalence of HCV infection with autoimmune diseases and the most common is chronic thyroiditis, which is an inflammatory disease that leads to hypothyroidism. These individuals have an increased level of antithyroid peroxidase antibody (anti TPO-Ab).

Objectives: To determine the prevalence of antithyroid antibody (ATA) in patients of HCV genotype 3a.

Material and method: Fifty HCV infected patients with genotype 3a were enrolled for this study that included 33 males and 17 females. After written informed consent, 3 ml blood of all the patients was obtained and ATA were detected by indirect immunofluorescence technique. These patients were divided into 3 groups, i.e. untreated 26 (52%), mid treated 17 (34%) and 7 (14 %) patients with hepatocellular carcinoma (HCC).

Results: Among the patients 17(51.5%) males and 7 (41.2%) females had ATA. Regarding different groups, 19 (73.1%) of untreated, 5 (29.4%) of mid treated and none of the patient in HCC group had ATA.

Conclusion: ATA was detected in a high percentage of patients with HCV genotype 3a. These antibodies were significantly high in untreated patients as compared to mid treated and HCC patients. Further, more males had these antibodies as compared to females.

Key words: Autoantibodies; Antithyroid antibody; ATA; HCV; Genotype 3a

Introduction

Worldwide, hepatitis C virus (HCV) infection is a major cause of liver disease. Acute HCV infection is commonly asymptomatic but can be accompanied by fatigue and jaundice while 75%–85% of HCV infected individual's progress to chronic state. About 150 million people globally have chronic HCV infection [1] whereas almost 3% of the world's population is chronic carrier of this virus. Chronic HCV infection is often characterized by steatosis, fibrosis and liver cirrhosis [2]. About 35% of chronic HCV symptomatic patients develop cirrhosis after 20 years of infection and a quarter of these cirrhotic patients develop hepatocellular carcinoma (HCC) after 30 years of infection. There are various associations of different subtypes of HCV e.g.

patients of genotype 1a have severe liver disease with poor prognosis while genotype 2a is found in healthy blood donors and are highly prevalent among patients with cryoglobulinemia whereas genotype 3 is associated with increased liver fibrosis and steatosis [3].

HCV infection is associated with autoimmune diseases. One of the common manifestations of HCV is thyroid disorders, characterized by increased level of circulating thyroid peroxidase (TPO-Ab) and thyroglobulin (Tg-Ab) antibodies [5]. Production of TPO-Ab is an initial sign of autoimmune thyroid disease (AITD) with an increased risk of hypothyroidism. Interferon suppresses tumors growth and modulates immune response. Therefore, it is used to treat various neoplastic, viral and autoimmune disorders [6]. IFN- α is being used successfully in clinical conditions, but due to immune enhancement or immune dysregulation, it has various side effects ranging from symptoms like influenza, severe hematological, neuropsychiatric and thyroid disorder [7].

Up to 15% of thyroid disorders have been reported in IFN- α treated HCV patients [6] and about 40% of them develop thyroid antibodies. The chances of thyroid dysfunctions in INF-treated HCV patients having TPO-Ab are about 50 times more as compared to 5.4% in HCV patients who do not have TPO-Ab [8]. Since HCV genotype 3a is the most prevalent genotype in Pakistan, therefore, the present study was performed to determine the prevalence of ATA in patients of HCV genotype 3a.

Material and Method

The study was approved by the “Ethical Review Committee and Research Board” of University of Health Sciences Lahore, Pakistan

Patients: The study included 50 HCV patients with 3a genotype and they were recruited from the Department of Gastroenterology, Sheikh Zaid Hospital, Lahore. Genotype of HCV and viral load was determined by PCR while HCC was diagnosed on liver histopathology. There were 33 (66%) males and 17 (34%) females and mean $\pm$ SD of age of males was 39.3 ± 9.6 years and 33.9 ± 8.0 years for females. These patients were divided into 3 groups, i.e. untreated, mid treated and patients with HCC. Out of 50 patients, 52% were untreated and 34% at the mid stage of INF

treatment (3 months) while 14% had HCC. Patients on multiple transfusion, autoimmune disorder and HCV patients of other genotypes were excluded from the study.

Screening of ATA: After an informed consent, three ml blood was collected from all the study subjects. After centrifugation, serum was separated and stored at -80°C. Determination of ATA was performed by indirect immunofluorescence technique using tissue labeled slides and positive control from the manufacturer (ORGENTEC Diagnostika GmbH).

Statistical analysis

Statistical analysis was performed using SPSS 17.0. Mean, $\pm$ SD of age, sex, treatment duration and ATA was calculated. Fisher's Exact Tests was applied to determine the p-value. A p-value of ≤ 0.05 was considered as statistically significant.

Results:

Among the 50 HCV patients of genotype 3a, ATA was detected in the sera of 24 (48.0%) patients while 26 (52.0%) did not have these antibodies. In the untreated group 19 (73.1%) had ATA and in the mid treated group 5 (29.4%) subjects had ATA whereas none of the subject had these antibodies in HCC group and on comparison there was statistically significant difference among the three groups ($p = 0.000$) (Table-1).

Regarding gender distribution, 17 (51.5%) males and 7 (41.2%) females had ATA. In the untreated group, 15 (71.4%) males and 4 (80%) females and in the mid treated group 2 (40.0%) males and 3 (25%) females, while in HCC group none of the subject had ATA and on comparison the difference between the groups was statistically significant ($p = 0.002$) (Table 2).

Discussion:

In the current study, age range of the subjects was 28-46 years with the mean $\pm$ SD of age was 37.5 ± 9.4 years. ATA was detected in 48% of the studied population, which is not in agreement with Tran et al (1993) [9] who documented ATA in 12.5% of HCV patients. The difference in the results could be due to specific genotype of HCV studied in the current study, whereas Trans et al (1993) [9] determined ATA in HCV patients but did not consider their genotype. The present study is also not in agreement with William et al (1998) [10], who detected ATA in

22.5% of females with recurrent pregnancy loss. The disagreement of two studies might be due to different clinical conditions of the subjects whose antibodies were detected.

The current study documented 71.3% of untreated HCV patients had ATA whereas Rocco et al (2001) [11] reported 10.3%, Marazuela et al (1996) [12] suggested 6.7%, Carella et al (2002)[6] detected in 10.2% and Antonelli et al (2004) [13] documented ATA in 38% of untreated HCV patients. This disagreement could be due to the inclusion of HCV patients of all genotypes and large number of patients i.e., 130 patients in the study of Rocco et al (2001), Marazuela et al (1996) [12] included 207 patients and Carella et al (2002) [6] included 72 patients whereas in the current study only 50 patients were included. The other probable reasons for disagreement could be the sensitivity of the diagnostic technique used for the detection of ATA as ELISA was used by Marazuela et al (1996) [12] whereas IF was used in the current study which is more sensitive. The other reason for the difference could be the specific HCV genotype of patients as well because only the current study included specific HCV genotype. Further, variability of ethnicity of the studied population could be another reason.

The present study documented ATA in 29.4% of mid treated HCV patients with genotype 3a which is in agreement with Carella et al (2001) [14] who detected ATA in 34.6% of the mid treated HCV patients. The genotype of HCV patients was not mentioned in these studies. Increased prevalence of ATA in untreated than mid treated patients may be due the effect of treatment but it should be investigated by conducting further studies.

However, in another study Carella et al (2002) [6] reported ATA as 13.4% in mid treated HCV patients which do not support the present study. This difference could be due to different diagnostic techniques as Carella et al (2002) [6] used ELISA while present study used IF technique for the detection ATA. Yet another study by Jadali (2013) [15] documented ATA in 10.3% of the mid treated HCV patients. It could be due to the variation of time interval at which ATA was detected i.e. in the previous study HCV patients of 6 months treatment interval were labeled as 'mid treated' whereas in the present study subjects of 3 months treatment interval were labeled as 'mid treated'.

In the present study, 51.5% of males and 41.2% of females had ATA. Tran et al (1993) [9], detected ATA in 31% of the females whereas they could not detect ATA in male subjects.

Similarly Marazuela et al (1996) [12], detected ATA in 14.8% of females as compared to 1% of males. The disagreement could be attributed to the difference in HCV genotypes as these researchers did not mention specific HCV genotype whereas current study had only HCV patients with genotype 3a.

High level of ATA in the untreated patients and in males suggested association of ATA with 3a genotype and male subjects. Another reason for this variation could be due to the technique selected for the detection of ATA i.e. IF is more sensitive technique compared to other assays, therefore high level of ATA was detected.

Conclusion:

ATA was detected in high percentage of HCV patients with genotype 3a. These antibodies were significantly high in untreated patients as compared to mid treated and HCC patients. Further, more males had these antibodies as compared to females.

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Table-1 Number, percentage and comparison of anti-thyroid antibodies among the three groups

Study group	Anti-thyroid antibodies			p-value
	Positive n (%)	Negative n (%)	Total n(%)	
Untreated	19 (73.1%)	7 (26.9%)	26 (100.0%)	0.000*
Mid treated	5 (29.4%)	12 (70.6%)	17 (100%)	
HCC	0 (0.0%)	7 (100.0%)	7 (100.0%)	
Total	24 (48.0%)	26 (52.0%)	50 (100.0%)	

n=number, %=percentage, * $p \leq 0.05$; statistically significant

183 Table-2: Number, percentage and comparison of anti-thyroid antibodies in male and female of
 184 three groups

Sex	Anti-thyroid antibodies				p-value
		Positive	Negative	Total	
		n (%)			
Male	Untreated	15 (71.4%)	6 (28.6%)	21 (100%)	0.002*
	Mid treated	2 (40.0%)	3 (60.0%)	5 (100%)	
	HCC	0 (0.0%)	7 (100.0%))	7 (100.0%)	
	Total	17 (51.5%)	16 (48.5%)	33 (100%)	
Female	Untreated	4 (80.0%)	1 (20.0%)	5 (100.0%)	0.101
	Mid treated	3 (25.0%)	9 (75.0%)	12 (100.0%)	
	HCC	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Total	7 (41.2%)	10 (58.8%)	17 (100.0%)	

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186 n=number, %=percentage, * $p \leq 0.05$; statistically significant