

Original Research Article

Anti-thyroid antibodies in patients of HCV genotype 3a

Abstract

Background: Hepatitis C virus (HCV) infection is the major cause of liver disease worldwide. Each year, 3 to 4 million people are infected with HCV. Acute infection of HCV is often asymptomatic but can present with fatigue and jaundice. About 80% of HCV infected individual's progress to chronic state. HCV infection is accompanied by an increased prevalence of autoimmune diseases. The most common phenomenon of HCV infection is chronic thyroiditis which is an inflammatory disease that leads to hypothyroidism and individual present with an increased level of circulating TPO-Ab.

Objectives: to determine the prevalence of ATA in patients of HCV genotype 3a.

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

Results: In this study 17(51.5%) males and 7 (41.2%) females had ATA. Further, 19 (73.1%) in the untreated group, 5 (29.4%) in the mid treated group and none of the patient in HCC group had ATA.

Conclusion: ATA were detected in high percentage of patients of 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 the major cause of liver disease. Acute infection of HCV is commonly asymptomatic but can be accompanied by fatigue and jaundice while 75%–85% of HCV infected individual's progress to chronic state. 130–150 million people globally have chronic hepatitis C infection [1]. 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]. It has been documented that 35% of the chronic HCV symptomatic patients develop cirrhosis after 20 years of infection and almost a quarter of these cirrhotic patients develop hepatocellular carcinoma (HCC) after 30 years of infection. Different subtypes of HCV

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

Chronic thyroiditis is an inflammatory disease that leads to hypothyroidism where thyroid functions are reduced [4]. It is often seen in middle-aged women but it can occur at any age. It affects 0.1-5% of all the adults in Western countries [4].

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. Anti-thyroid antibodies (ATA) lead to autoimmune thyroiditis (AT) and they have high level of CXCL10, particularly if they have hypothyroidism [5]. TPO-Ab is an initial phase of autoimmune thyroid disease (AITD) and patients have an increased risk of hypothyroidism and patients of AT. 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 up to severe hematological, neuropsychiatric and thyroid disorder [7].

The correlation between IFN- α and thyroid diseases was observed during treatment of carcinoid tumors and breast cancer patients. High prevalence of thyroid disorder (up to 15%) has been reported in IFN- α treated HCV patients [6] and up to 40% developed thyroid antibodies and 1-35% of thyroid dysfunction. The chances of developing thyroid dysfunction in INF-treated HCV patients having TPO-Ab is about 50 times more as compared to 5.4% in HCV patients who do not have TPO-Ab [8]. Therefore, present study was performed to determine the prevalence of ATA in patients of HCV genotype 3a.

Material and Method

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

Patients: The study included 50 HCV patients of 3a genotype and they were recruited from the Department of Gastroenterology, Sheikh Zaid Hospital, Lahore. There were 33 (66%) males and

17 (34%) females and mean $\pm$ SD of age of male was 39.3 ± 9.6 years and 33.9 ± 8.0 years of females. These patients were divided in to 3 groups i.e. untreated, mid treated and patients of HCC. Out of 50 patients 52% patients were untreated and 34% patients were at the mid stage of INF treatment (3 months) while 14% were 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 to the subject, three ml blood was collected and after centrifugation their serum was separated. Determination of ATA was performed by indirect immunofluorescence technique.

Statistical analysis

Statistical analysis was performed on SPSS 17.0. Mean, $\pm$ SD, range of age, sex, treatment duration and ATA was calculated. Pearson Chi-square test and Fisher's exact test was applied to determine the p-value. A p-value of ≤ 0.05 was considered statistically significant.

Results:

Fifty patients of HCV genotype 3a were included in this study. Among these, 24 (48.0%) patients had ATA while 26 (52.0%) did not have these antibodies. In the untreated group 19 (73.1%) n and in 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 groups ($p = 0.000$) (Table-1).

In the current study, 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 ($p\text{-value} = 0.002$) (Table 2).

Discussion:

Age range of the subjects was 28-46 years with the mean $\pm$ SD of age as 37.5 ± 9.4 years. In the current study 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 HVC patients. The difference in results could be due to specific genotype of HCV patients included in the current study whereas Trans et

al (1993) [9] determined ATA in HCV patients but they were not of specific genotype. Present study is also not in agreement with the 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 where antibodies were detected.

The current study documented that 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% and 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 large number of patients i.e., 130 included in the study of Rocco et al (2001), Marazuela et al (1996) [12] included 207 patients and Carella et al (2002) [6] included 72 HCV patients whereas in the current study 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 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 patients of HCV 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 this 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 patients of HCV. It could be due to the variation of time at the ATA was detected i.e. in the previous study patients of 6 months treatment interval were labeled as 'mid treated' whereas in the present study subjects of 3 month 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 of 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 assay 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 were detected in high percentage of patients of 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.

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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