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ORIGINAL ARTICLE

HLA Status and Antithyroid Autoantibodies in Egyptian Patients with Chronic Hepatitis C Virus Infection

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ABSTRACT

Background/Aim: The role of hepatitis C virus (HCV) has been demonstrated in many autoimmune diseases, including the autoimmune thyroid disease. However, there is paucity of information about the prevalence of extrahepatic autoimmune phenomenon in HCV-infected patients from Egypt and developing countries. This study checked the prevalence of antithyroid autoantibodies in patients with chronic HCV infection and their possible relation to the human leukocyte antigen (HLA) status.

Patients and methods: Sera from 147 consecutive patients (75 males and 72 females) with chronic HCV infection at the Mansoura University Hospitals, Egypt during 2008 to 2009, were analyzed for antithyroid antibodies (group 1). A total of 126 anti-HCV positive patients without antithyroid antibodies were enrolled as controls (group 2).

Thyroid microsomal and thyroglobulin autoantibodies were determined by the hemagglutination tests**.** IgG type anti-GOR were measured using an ELISA assay. HLA-A,-B, -C and -DR were determined using the standard complement-dependent microdroplet lymphocyte cytotoxicity test.

Results: Antithyroid antibodies were detected in 21 HCV RNA positive patients (group 1; 18 females, 3 males) and remaining 126 patients (group 2; 72 males and 54 females). The prevalence of anti-GOR antibodies was significantly higher in patients of group 1 compared to that of group 2.

A statistically significant difference was observed regarding anti-GOR antibodies in group 1 ($p < 0.001$), antithyroid autoantibodies among the females ($p < 0.001$). HLA-A2 antigen was prevalent in group 1 ($p < 0.05$).

Conclusion: Our data revealed that HLA-A2 may be regarded as an immunologic risk factor for the development of antithyroid autoantibodies in patients with chronic HCV infection and these autoantibodies should be evaluated prior to initiating IFN-α therapy.

Abbreviations: HCV—Hepatitis C virus; IFN—Interferon; HLA—Human leukocyte antigen; ALT—Alanine aminotransferase.

Keywords: Antithyroid autoantibodies, HCV, HLA antigens.

INTRODUCTION

The role of hepatitis C virus (HCV) has been demonstrated in many autoimmune diseases, such as sailadenitis, type II cryoglobulinemia, autoimmune idiopathic thrombocytopenic purpura and thyroid diseases.[1] High prevalence of autoimmune thyroid disease has been reported in older women[2], however, there is lack of consensus about this.[3] Treatment with interferon (IFN) induces thyroid disease in 1 to 5% of patients that are usually asymptomatic.[4] In addition, studies have shown IFN-related symptomatic thyroid diseases in 3 to 15% of cases.[5] Although most of the side effects of IFN treatment for chronic HCV infection are reversible,[6] the development of irreversible autoimmune disease may lead to discontinuation of IFN therapy.

The presence of antithyroid autoantibodies has been suggested as a risk factor for IFN-related thyroid dysfunction and may represent a predictor of on-treatment hypothyroidism.[7,8]

This work was designed to study the prevalence of antithyroid autoantibodies in patients with chronic HCV infection and their possible relation to the human leukocyte antigen (HLA) in Egyptian patients.

PATIENTS AND METHODS

A total of 147 patients with chronic HCV infection were enrolled in his study. These patients attended the Mansoura University Hospitals, Egypt for IFN therapy during 2008-2009 (group 1). A total of 126 anti-HCV antibody positive

but negative for antithyroid antibodies were enrolled as control (group 2).

The inclusion criteria were the following: (1) The presence of HCV RNA in the sera, (2) elevation of serum ALT over 40 IU/liter (upper normal limit) for six months or longer, (3) liver biopsy suggestive of chronic hepatitis, (4) exclusion of other possible causes of chronic liver disease, (5) no evidence of ongoing infection with HBV or HIV and (6) no previous history of IFN therapy. The detection of HCV RNA in sera was performed by the nested polymerase chain reaction (PCR) technique.

Thyroid microsomal and thyroglobulin autoantibodies were determined by the hemagglutination tests (Thymune M and T, Wellcome Diagnostics). Antithyroperoxidase autoantibodies were measured by the radioimmunoassay (Dyno test, Henning Laboratories; upper normal limit 100 U/ml).

IgG type anti-GOR were measured using an ELISA assay in a microwell plate with the semisynthetic peptide GOR-2 bound to the solid phase in a standard procedure.[9] HLA-A,-B, -C and -DR were determined using the standard complement-dependent microdroplet lymphocyte cytotoxicity test.[10]

Statistical analysis was done using SPSS version 15 software (Chicago, IL, USA). All results were expressed as mean ± SD. All statistical significance between means was calculated by the student's t-test. Differences between proportions were assessed by χ^2 test. A p-value of < 0.05 was considered to be statistically significant.

RESULTS

Out of 147 patients in group 1, antithyroid antibody was detected in 21 patients. These 21 patients with HCV infection and antithyroid antibody were compared with 126 HCV-infected antithyroid negative patients of group 2.

As described in the previous section, 21 patients of group 1 expressed antithyroid antibodies (Table 1). Nine of these patients expressed thyroid microsomal antibodies, and four and eight patients were positive for antithyroid antibodies and thyroperoxidase antibodies respectively.

Table 1: The prevalence of antithyroid antibodies and anti-GOR antibodies in hepatitis C virus-infected subjects

Parameters	*Group 1 (Total 21)*	*Group 2 (Total 147)*
Anti-GOR antibodies	18 (85.71%)	23 (15.64%)
Anti-thyroid antibodies	42.85%*	6.12%
Thyroid Microsomal Abs	19.04%*	2.72%
Antithyroglobulin Abs	38.09%*	5.44%

*$p < 0.001$, compared to other group 2

Anti-GOR antibody was detected in 18 patients of group 1 with a female prevalence (3 male; 15 female patients). On the contrary, anti-GOR antibody was detected in only 23 of 147 patients of group 2. The prevalence of anti-GOR antibody was significantly higher in HCV-infected patients expressing antithyroid antibodies compared to those negative for these ($p < 0.05$).

HLA-A2 haplotype was significantly higher in patients of group 1 (76%) compared to those of group 2 (32%) ($p < 0.05$) (Table 2). An elaborative description of HLA typing that included different HLA haplotype of patients of group 1 has been shown in Table 3.

DISCUSSION

A high prevalence of antithyroid autoantibodies was observed in patients with chronic HCV infection before the initiation of IFN therapy. Pawlotsky et al found antithyroid antibodies in five of 61 patients (7%) with chronic HCV infection.[11] While, others found a lower prevalence (3%) of these antibodies in their patients.[12] In fact, considerable

Table 2: HLA distribution among hepatitis C virus-infected patients with and without antithyroid antibodies

HLA	*Group 1 (21) HCV+ antithyroid autoantibodies (+ve)*	*Group 2 (126) HCV+ antithyroid autoantibodies (-ve)*	*p-value*
A2	16 (76.19%)	41 (32.53%)	< 0.05
A11	6 (28.57%)	35 (27.77%)	NS
A24	11 (42.30%)	51 (40.47%)	NS
A26	1 (4.76%)	7 (5.55%)	NS
A31	5 (23.8%)	28 (22.22%)	NS
B7	12 (57.14%)	68 (53.96%)	NS
B39	3 (14.28%)	20 (15.87%)	NS
B44	1 (4.76%)	4 (3.17%)	NS
B46	4 (19.04%)	26 (20.63%)	NS
B51	4 (19.04%)	25 (19.84%)	NS
B52	4 (19.04%)	26 (20.63%)	NS
B56	1 (4.76%)	5 (3.96%)	NS
B59	3 (14.28%)	19 (15.07%)	NS
B60	1 (4.76%)	7 (5.55%)	NS
B61	9 (42.85%)	55 (43.65%)	NS
Cw1	7 (33.33%)	42 (33.33%)	NS
Cw3	9 (42.85)	51 (41.26%)	NS
Cw4	1 (4.76%)	5 (3.96%)	NS
Cw7	8 (38.09%)	49 (38.88%)	NS
DR1	8 (38.09%)	48 (38.09%)	NS
DR2	6 (28.57%)	35 (27.77%)	NS
DR4	6 (28.57%)	36 (28.60%)	NS
DR8	6 (28.57%)	35 (27.77%)	NS
DR9	2 (8.33%)	12 (9.52%)	NS
DR11	1 (4.76%)	7 (5.55%)	NS
DR12	1 (4.76%)	5 (3.96%)	NS
DR13	1 (4.76%)	7 (5.55%)	NS
DR14	1 (4.76%)	5 (3.96%)	NS

NS – not significant

Table 3: HLA frequencies in HCV positive patients with antithyroid autoantibodies

Case	*Age*	*Sex*	*HLA*							
1	21	F	A2		B7	B51	DR1	DR4	Cw1	Cw3
2	23	F	A2	A24	B7	B7	DR1		Cw1	Cw4
3	34	F	A2	A24	B46	B61	DR2	DR4	Cw3	
4	33	M	A2	A24	B39	B61	DR4	DR6	Cw3	
5	40	F	A11	A24	B7	B59	DR1	DR2	Cw7	
6	34	F	A24	A26	B52	B51	DR1	DR11	Cw1	
7	29	F	A11	A24	B7	B60	DR1	DR12	Cw7	
8	28	F	A2	A11	B7	B52	DR2	DR4	Cw1	
9	36	F	A31	A11	B7	B51	DR8	DR9	Cw3	
10	38	F	A2	A31	B46	B59	DR4	DR8	Cw1	Cw7
11	35	F	A2	A24	B7	B61	DR6	DR1	Cw1	Cw3
12	23	M	A2	A24	B61	B56	DR6	DR8	Cw3	
13	24	F	A2	A31	B7	B61	DR8	DR13	Cw7	
14	35	F	A2	A31	B44	B46	DR2	DR14	Cw7	
15	29	F	A2	A31	B39	B61	DR8		Cw1	Cw7
16	36	F	A2	A11	B46	B51	DR6	DR8	Cw3	
17	25	F	A11	A24	B7	B59	DR4	DR6	Cw3	
18	22	F	A2	A24	B7	B52	DR1		Cw7	
19	39	F	A2		B52	B61	DR2		Cw7	
20	30	F	A2	A24	B39	B61	DR2		Cw1	
21	40	M	A2		B7	B61	DR1	DR9	Cw3	

variations of prevalence of these antibodies, ranging from 1 to 30%, have been reported in literatures.[13-15]

In this study, 21 out of 147 patients (14.28%) had anti-thyroid autoantibodies with a high prevalence in females. This supports the notion that many autoimmune thyroid disorders are common in females.

Microsomal antibodies (Abs), antithyroglobulin Abs, thyroperoxidase Abs were positive in 6.12, 2.72 and 5.44% patients of group 1 respectively. The observed differences between the prevalence in different series may be related to HCV genotype, duration of HCV infection, the laboratory method of immunoassay, and their geographic origin and age. The prevalence increased with age in most cases. An overall 13.7% prevalence of antithyroid peroxidase antibodies in 60 to 80-year-old subjects with a maximum of 22% in the seventh decade has already been reported.[16] Identification of such autoantibodies in patients with chronic HCV may have prognostic significance before the initiation of IFN therapy, as described before.[17]

Anti-GOR is a common finding in patients with well-documented chonic HCV infection[9,18] and it has been suggested that anti-GOR may reflect HCV-induced autoimmunity.[9,19]

High prevalence of anti-GOR antibodies (p-value < 0.001) were demonstrated in patients of group 1 that exhibited both anti-HCV and antithyroid autoantibodies.

The presence of anti-GOR antibodies in the former group supports suggestions claiming that the presence of antithyroid antibodies is not an incidental finding and these may have close association with HCV infection. Despite a lack of a detailed characterization of the GOR protein, partial sequence homology exists between the GOR protein and residues 4 to 20 of the nucleocapsid protein of HCV and the anti-GOR may, thus, be the antibody induced by the HCV infection that cross-reacts with a host peptide.[20]

Despite the absence of cross-reactivity between the antithyroid autoantibodies and anti-HCV antibodies, researchers suggested the possibility that HCV and thyroid proteins may share a partial sequence in a few amino acid segments.[21,22] It has been reported that 67.1% of patients with hyperthyroidism were positive for HLA-A2 and 59.2% of patients with Hashimoto's thyroiditis were also positive for HLA-A2; an observation suggesting that HLA-A2 is significantly associated with the presence of autoimmune thyroid disorders.[23]

In the present study, HLA-A2 was present in 76.19% of patients who developed antithyroid autoantibodies in patients with chronic HCV infection. Therefore, HCV may function as a trigger for the evolution of antithyroid autoantibodies in HLA-A2 positive cases.

An increased frequency of HLA-B46 has been reported in autoimmune thyroid disease during or after IFN therapy.[24] But in the present study, HLA-B46 was present in 19.04% of patients with autoantibodies and was not statistically significant when compared with the antibody-negative group ($p < 0.005$). Among the associations of HLA class I alleles, HLA-A2 and B46 are known to be in linkage disequilibria in the studied population.[23]

The presence of HLA-DR1 was associated with a lower frequency of immune manifestations in patients with chronic HCV infection.[23] However, in the present study, HLA-DR1 did not seem to be protective against the development of HCV-related autoantibody production because HLA-DR1 frequency was 38.09% in patients with anti-thyroid autoantibody.

Our data revealed that HLA-A2 may be regarded as an immunologic risk factor for the development of antithyroid autoantibodies in patients with chronic HCV infection and considering their prognostic significance, these autoantibodies should be evaluated prior to initiating IFN-α therapy.

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