Thyroid and Celiac Autoantibodies in Patients with Chronic Hepatitis C

Kronik Hepatit C Hastalarında Tiroid ve Çölyak Otoantikorları

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ABSTRACT Objective: A large number of studies report an association between chronic hepatitis C virus (HCV) infection with autoimmune disorders such as mixed cryoglobulinemia, lichen planus, thyroiditis and celiac disease. Moreover, the association between autoimmune thyroid diseases and celiac disease has been well-established for many decades. The aim of this study was to investigate anti-transglutaminase (ATTG), anti-thyroglobulin (ATG) and anti-thyroid peroxidase (ATPO) autoantibodies and the role of interferon-alpha (IFN-α) treatment on their levels in chronic HCV (CHCV) infected individuals. **Material and Methods:** This study included 170 individuals; 95 had HCV infection and 75 were healthy controls. The patients were divided into two groups. One group included 42 patients with CHCV infection receiving IFN-α or pegylated-IFN-α plus ribavirin for at least 52 weeks and the other group included 53 patients with HCV infection with no treatment. ATTG, ATG and ATPO levels of the patient groups were measured. **Results:** ATTG positivity was higher in patients with HCV infection (n= 95) (21.5%) than in controls (n= 75) (4%) (p= 0.001) with a higher rate among patients who did not receive treatment. ATTG positivity was 4% in controls, 11.9% in patients on treatment (p= 0.104) and 28.3% in patients who did not receive treatment (p= 0.0001). There was no significant difference in thyroid autoantibodies between the patient group and controls and between each patient subgroup and controls. There was also no significant correlation between ATTG and thyroid autoantibodies. **Conclusion:** ATTG may be suggested to be more significantly associated with HCV than thyroid autoantibodies and patients with HCV infection should be examined for celiac disease symptoms although they do not receive treatment and celiac disease autoantibodies should be investigated when necessary.

Key Words: Hepatitis C, chronic; celiac disease; thyroid gland; autoantibodies

ÖZET Amaç: Kronik hepatit C virüsü (HCV) enfeksiyonu ile mikst kriyoglobülinemi, liken planus, tiroidit ve çölyak hastalığı gibi otoimmün hastalıkların birlikteliği çok sayıda çalışmada bildirilmiştir. Bunun yanı sıra, otoimmün tiroid hastalıkları ile çölyak hastalığı arasındaki ilişki bilinmektedir. Kronik hepatit C virüsü (KHCV) enfeksiyonu olanlarda anti-transglutaminaz (ATTG), anti-tiroglobülin (ATG) ve anti-tiroit peroksidaz (ATPO) otoantikorlarını ve interferon-alfa (IFN-α) tedavisinin bu antikorların düzeyleri üzerindeki etkisini araştırmayı amaçladık. **Gereç ve Yöntemler:** Çalışmaya 95 HCV hastası ile 75 sağlıklı kontrol olmak üzere toplam 170 kişi dahil edildi. Hastalar, IFN-α ya da pegile-IFN-α + ribavirin tedavisini en az 52 hafta süreyle almış olan 42 KHCV hastası ve HCV enfeksiyonu olup, tedavisiz takip edilen 53 hasta olmak üzere iki gruba ayrıldı. Çalışma grubunun ATTG, ATG, ATPO değerlerine bakıldı. **Bulgular:** HCV enfeksiyonu olan hastalarda (n= 95) (%21.5) ATTG pozitifliği, kontrol grubundaki orana (n= 75) (%4) kıyasla yüksek bulundu (p= 0.001); oran, tedavi almayan HCV enfeksiyonlu hastalarda, alanlara görece daha yüksekti. ATTG pozitiflik oranı kontrol grubunda (n= 75) %4 iken, tedavi alan HCV grubunda (n= 42) %11.9 (p= 0.104), tedavi almayan HCV grubunda (n= 53) ise %28.3 idi (p= 0.0001). Tüm hasta grubu kontrol ile karşılaştırıldığında ve hasta grubu tedavi alan ve almayan olarak alt gruplara ayrılıp kontrol ile karşılaştırıldığında, ATG ve ATPO oranları arasında istatistiksel olarak anlamlı bir fark saptanmadı. ATTG ile tiroid otoantikorları arasında herhangi bir ilişki saptanmadı. **Sonuç:** KHCV enfeksiyonu ile ATTG arasında anlamlı bir ilişki bulunurken, ATG ve ATPO ile anlamlı bir ilişki saptanmadı. KHCV enfeksiyonu olan hastalar interferon tedavisi almasalar bile çölyak ile ilişkili semptomlar açısından değerlendirilmeli, semptomatik olgularda çölyak ile ilişkili otoantikorlar araştırılmalıdır.

Anahtar Kelimeler: Kronik hepatit C; çölyak hastalığı; tiroid; otoantikorlar

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Hepatitis C virus (HCV) infection does not cause only chronic liver disease but also stimulates autoimmune responses and may be associated with many autoimmune diseases such as antibody development related to extrahepatic systems, mixed cryoglobulinemia, lichen planus, thyroiditis and celiac disease (CD).[1,2] Standard treatment for chronic HCV (CHCV) infection is the combination of IFN-α with ribavirin.[3-4] IFN-α has antiviral as well as immunomodulatory effects. Thus, it is contraindicated in patients with diseases known to be exacerbated by autoimmune hepatitis or interferon treatment.[5]

CD is a chronic autoimmune disease characterized by malabsorption resulting from inappropriate immune responses to gluten ingested through diet.[6] Clinicians are increasingly utilizing noninvasive serologic tests for the diagnosis and screening of CD. The sensitivity of ATTG in children and adults is 98% and 96%, respectively.[7] Detection of ATTG by enzyme-linked immunosorbent assay (ELISA) is highly sensitive and specific with a positive predictive value of 90% and a negative predictive value of 98%.[8] CD was reported to be activated during IFN-α treatment in patients with HCV infection, but it is not clear whether IFN-α treatment or HCV infection itself play a role in the development of autoantibodies to CD.

Several studies have revealed that thyroid autoantibody levels are high in patients with HCV infection and are increased during IFN-α treatment.[9-11]

However, the incidence of CD has been reported to be increased in patients with autoimmune thyroid disease.[12-14] There is no evidence for the association of CD with thyroid autoantibodies, in patients with HCV infection.

The aim of this study was to investigate the role of HCV infection and IFN-α treatment on ATTG and thyroid autoantibodies and the association of ATTG with thyroid autoantibodies in patients with HCV infection, which was previously reported in the literature.

MATERIAL AND METHODS

STUDY POPULATION

The study included 170 individuals, 95 patients with HCV infection followed-up in our clinic between 2003 and 2006 and 75 anti-HCV negative healthy controls. The patients were assigned into two groups; one group included 42 patients with CHCV infection who received IFN-α or pegylated IFN-α plus ribavirin for at least 52 weeks and the other group included 53 patients with HCV who received no treatment (IFN-α or pegylated IFN-α plus ribavirin). The former group included patients who were positive for HCV RNA and who had liver biopsy results suggestive of chronic hepatitis. Patients with HCV accompanied by an autoimmune disease and a co-infection with HBV were not included in the study.

The control group consisted of 75 adults who had no previously diagnosed CD or thyroid disease.

Informed consent was obtained from all patients and controls. The study protocol was approved by the Hospital Ethics Committee.

LABORATORY ANALYSIS

Microparticle enzyme immunological assay (MEIA) (AxSYM, Abbott Laboratories, Wiesbaden, GERMANY) was used to measure anti-HCV, anti-thyroglobulin and anti-thyroid peroxidase levels and ELISA was used to measure anti-transglutaminase levels. ElectroChemiLuminescense immunoassay (ECLIA) (COBAS, Roche Diagnostics, USA) was used to measure thyroid hormones, B12 and folate levels. HCV RNA was measured by Cobas Amplicor HCV monitor test v 2.0 (Roche Diagnostics, USA).

Statistical Analysis

Data were evaluated with SPSS (ver.11.5). Statistical analyses were made with T test, Chi-square test, Mann-Whitney U, One-way ANOVA and Kruskal-Wallis tests where appropriate. $P< 0.05$ was considered significant.

RESULTS

Table 1 shows demographic features and laboratory results of patient and control groups. There were

TABLE 1: Clinical and laboratory findings in the study population.

Variable	CHCV with IFN n= 42	CHC without IFN n= 53	Controls n= 75	p
Age (years)	53.6 ± 10.8	55.2 ± 13.3	51.7 ± 11.2	0.250
Male/Female gender	14/28	28/25	36/39	0.250
HCV infection duration (months)	51.6 ± 36.3	18.5 ± 25.8	-	<0.01
Hb (g/dL)	15.3 ± 14.1	13.8 ± 1.6	13.3 ± 1.8	0.250
Htc (%)	38.2 ± 5.1	40.5 ± 4.1	39.8 ± 4.5	0.062
Serum iron (mg/dL)	101.6 ± 47.5	95.8 ± 47.7	75.4 ± 41.2	0.007
Ferritin (ng/mL)	239.8 ± 261.1	123.7 ± 121.4	78.7 ± 90.6	0.0001
AST(U/L)	44.3 ± 35.2	48 ± 36	19.9 ± 5	0.0001
ALT(U/L)	45.1 ± 40.2	57.5 ± 48.2	21.9 ± 11.8	0.0001
Free T3 (pmol/L)	4.9 ± 2.4	4.9 ± 5.4	5 ± 0.6	0.407
Free T4(pmol/L)	14.1 ± 5	14.5 ± 5.4	14.7 ± 4.3	0.767
TSH (mIU/mL)	2.8 ± 8.8	2.9 ± 9.7	2.5 ± 7.2	0.384
Vitamin B12 (pg/mL)	292.5 ± 102.5	430.6 ± 275	380 ± 265	0.003
Folic acid (ng/mL)	10.3 ± 4.3	9.2 ± 2.9	28.1 ± 157.3	0.729
HCV RNA positivity n (%)	23 (54.8%)	39 (73.6%)	-	<0.001
ATPO (IU/mL) (IU/mL)	8 (19 %)	8 (15.1%)	12 (16%)	0.866
ATG (IU/mL) (IU/mL)	28 (66.7%)	41(77.4%)	54 (72%)	0.510
ATTG (U/mL) (IU/mL)	5 (11.9 %)	15 (28.3 %)	3 (4%)	**0.0001**

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, HCV: Hepatitis C virus,
ATTG: Anti-transglutaminase, ATG: Anti-thyroglobulin, ATPO: Anti-thyroid peroxidase, TSH:Thyroid stimulant hormone.

no differences between the groups for age, autoimmune thyroid disease (familial history of thyroid disease and history of anti-thyroid treatment) and symptoms likely to be associated with celiac disease (nausea, vomiting and weight loss). Results of thyroid function tests were similar in all groups. The mean duration of treatment was 13.1 ± 6.9 months for the patients on treatment. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum iron and ferritin levels were significantly lower in controls than in other groups (p= 0.0001, p= 0.0001, p= 0.007, p= 0.0001, respectively). Serum vitamin B12 levels were lower in HCV patients who received treatment than in the other two groups (p= 0.003).

The rate of ATTG positivity was higher in HCV patients (n= 95) than in controls (n= 75) (21.5% vs. 4%) (p= 0.001). The rate of ATTG positivity was significantly higher in HCV patients who did not receive treatment (p= 0.0001). ATTG was positive in 4% of controls, 11.9% of CHCV patients who received treatment (n= 42) and 28.3% of HCV patients who did not receive treatment (n= 53) (Table 1). The difference between ATTG positive and ATTG negative patients for celiac-related symptoms was not significant.

There was no significant difference in ATPO and ATG autoantibody positivity between HCV patients and controls (p= 0.883 and p= 0.994 respectively) and between all three groups (p= 0.866 and p= 0.510 respectively) (Table 1). There was no significant relation between ATTG positivity and ATPO (p= 0.464) and ATG (p= 0.880) positivities. However, the association of ATPO with ATG positivity was significant as expected (p= 0.002).

DISCUSSION

We found that ATTG positivity was higher in HCV patients than in controls. Fine et al. compared patients with HCV infection, patients with other liver diseases and healthy controls and found that HCV infection was the most common liver disease accompanying CD[1]. Another study revealed that 1.3% of CHCV patients had silent CD and that this prevalence was three times higher than in the control group and in the geographical population with-

out a significant difference.[15] However, in the present study, ATTG was positive in a significantly higher rate of HCV patients compared to controls (p= 0.001).

Using highly sensitive tests such as anti-endomisium antibody and tissue transglutamase antibody tests, the studies on Western societies revealed that the prevalence of CD was over 1%, which was attributed to the asymptomatic course of the disease.[6] Reports from Turkey suggest that the prevalence of gluten sensitivity ranges from 0.02% to 0.7%.[16,17] In fact, the prevalence is thought to be higher than reported due to the high frequency of asymptomatic disease.

In the present study, ATTG was positive in 4% of the control group and 21.5% of the HCV patients. Most of the patients were asymptomatic and there was no significant relation between ATTG positivity and symptoms of CD. Serologic tests such as ATTG or endomysial antibody have a high sensitivity and specificity but endoscopic evaluation and small bowel biopsy are the gold standard for CD diagnosis.[6] Endoscopic evaluation was offered to patients who had ATTG positivity for CD diagnosis, but all patients rejected. Therefore, ATTG positivity rates are not indicative of CD prevalence due to asymptomatic ATTG positivity. In this study a 50% increase in ATTG levels was associated with lower bone mineral density of the hip, lower hemoglobin levels, decreased weight, lower cholesterol, and higher blood glucose.[18]

A significantly higher rate of ATTG positivity in HCV patients who did not receive treatment and lacked CD activation during treatment suggested that HCV infection itself rather than interferon treatment played a more important role in the etiology of CD autoantibody positivity. Consistent with the results of this study, Fine et al. suggested that HCV infection rather than IFN-α was associated with an increased prevalence of CD in a patient population.[1]

There have been case reports about the activation of CD during IFN-α treatment.[19] IFN-α directly exerts its effects on the tissues and modifies lymphocyte population and cytokine production, thereby interacts with the immune system and may cause autoimmune disorders. IFN-α converts T-Helper2 to T-Helper1 and regulates the cytotoxycity of T cells and NK cells.[20] Ribavirin increases the immune response through TH1 cytokine and decreases the TH2 response.[21] Therefore, treatment with IFN-α or IFN-α plus ribavirin activates TH1 cells, which play a role in the pathogenesis of CD and may activate CD. Durante-Mangoni E et al reported that IFN-α precipitated silent CD in CHCV patients and induced symptoms similar to those of CD such as diarrhea, anemia and extreme weight loss in CHCV patients with ATTG positivity before treatment.[15] In this study, none of the CHCV patients had symptoms of CD and we did not observe CD activation during IFN-α treatment.

IFN-α treatment was shown to contribute to autoimmune diseases in HCV patients.[22] It is not known whether HCV itself is a potent stimulator of the autoimmune system. There have been many relevant studies in the literature. One study revealed that anti-TPO was positive in 20% of CHCV patients, which was higher than in controls and in another study the rate of anti-TPO positivity was 14.7% in CHCV patients and it was shown to be similar to that in the normal population.[23,24] Floreani et al did not find a relation between thyroid autoantibody positivity and HCV.[25] In the present study, there were no differences in thyroid autoantibody levels between patient and control groups and between all three groups. The prevalence of CD among autoimmune thyroid disorders has been reported to range from 2% to 4.8% in studies looking for a correlation between the two disorders.[12,14,26,27] We did not find any correlation between thyroid autoantibody levels and ATTG positivity. Our study groups consisted of HCV patients and healthy controls. However, we could not find any correlation most likely due to the low number of seropositive patients for statistical evaluation.

CONCLUSION

Based on our findings, ATTG was positive in 21.5% of HCV patients and at a higher rate in those who did not receive treatment. However, most ATTG

positive cases were asymptomatic, which may be attributed to the presence of latent or silent CD. Therefore, we can suggest that CD symptoms should be investigated not only before interferon treatment but also in HCV patients who are not given treatment; in addition, in asymptomatic cases CD autoantibodies should be investigated in individual cases.

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