The Relationship Between Parvovirus B19 and Hashimoto's Thyroiditis

Hashimoto Tiroiditi ile Parvovirüs B19 Arasındaki İlişki

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ABSTRACT Objective: Hashimoto thyroiditis (HT) is among the autoimmune diseases of the thyroid gland. Acute parvovirus B19 infection is suggested to be involved in the pathogenesis of some HT cases. The aim of this study was to test IgM and IgG antibodies against Parvovirus B19 in adult patients with HT. **Material and Methods:** The study was run in the Department of Internal Medicine, İstanbul University, İstanbul, Turkey between April 2010 and March 2011. Serum samples drawn from 54 adult patients with HT and from 35 age-matched controls were tested for IgM and IgG antibodies against parvovirus B19. The chi-square test and unpaired t-test were used for the comparison between the groups. **Results:** Anti-parvo virus IgM antibodies were positive in 6 (11.1%) patients and 3 (5.7%) controls while anti-parvo virus IgG antibodies were present in 14 (25.9%) patients and 8 (22.9 %) controls. There was no significant difference between the patient and control groups in terms of antibody positivity (p=0.409, p=0.478). **Conclusion:** Although the prevalence of antiviral antibodies were higher in patients with HT compared to those in the control group, the difference was not statistically significant. This was attributed to the small number of patients. Nevertheless, this finding may reflect a viral contribution in the pathogenesis of HT.

Key Words: Parvovirus B19, human; Hashimoto disease; autoimmune diseases

ÖZET Amaç: Hashimoto tiroiditi (HT), tiroid bezinin otoimmün hastalıklarından biridir Akut parvovirüs enfeksiyonunun Hashimoto tiroiditi olan bazı vakaların patogenezinde rolü olduğu ileri sürülmektedir. Bu çalışmanın amacı, Hashimoto tiroiditli erişkin hastalarda parvovirus B 19 immün globülin (Ig) M ve G antikorlarının varlığını araştırmaktır. **Gereç ve Yöntemler:** Bu çalışma, Nisan 2010-Mart 2011 tarihleri arasında İstanbul Üniversitesi İstanbul Tıp Fakültesi İç Hastalıkları Anabilim Dalı'nda yapıldı. Hashimoto tiroiditi olan 54 hastadan ve yaş açısından eşleştirilmiş 35 kontrol olgusundan kan alındı. Alınan örneklerde parvovirüs B19 Ig M ve Ig G antikorları araştırıldı. İstatistiksel analizler için ki- kare testi ve bağımsız örneklem t-testi, gruplar arası karşılaştırmalarda kullanıldı. **Bulgular:** Anti-parvovirüs Ig M antikoru 6 (%11,1) hastada ve 3 (%5,7) kontrol olgusunda, Ig G antikoru 14 (%25,9) hastada ve 8 (%22,9) kontrol olgusunda pozitif saptandı. Antikor pozitifliği açısından kontrol ve hasta grupları arasında anlamlı bir fark yoktu (p=0,409, p=0,478). **Sonuç:** Anti-parvovirüs antikor pozitifliği, kontrollerle karşılaştırıldığında HT olan hastalarda daha fazla saptanmasına rağmen, muhtemelen hasta sayısının az olması nedeniyle istatistiksel olarak anlamlı değildi. Yine de bu bulgu, HT patogenezinde viral bir sebebin varlığına işaret edebilir.

Anahtar Kelimeler: Parvovirüs B19, insan; Hashimoto hastalığı; otoimmün hastalıklar

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Hashimoto's thyroiditis (HT) is the most common and extensively studied organ-specific autoimmune disorder in humans.[1,2] It is characterized by diffuse lymphocytic infiltration of the thyroid gland, elevated levels of the serum antithyroid antibodies (ATA), clinical

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evidence of goitrous or atrophic gland and frequent thyroid dysfunction in varying degrees.[2] The disease has been suggested to be the clinical expression of cell mediated immunity leading to the destruction of thyroid cells.

The pathogenetic mechanisms of HT are not completely defined.[2] The immune involvement in HT is demonstrated by many findings: (1) activation of $CD4^+$ T lymphocytes specific for thyroid antigen, (2) expression of major histocompatibility-complex (MHC) class II proteins induced by interferon-γ, (3) stimulation of autoreactive B cells by self-reactive $CD4^+$ T cells to secrete antithyroglobulin antibody, and (4) cytokine-regulation by apoptotic pathways.[2,3] Environmental agents such as virus infection have been investigated as potential etiologies of HT.[4,5] Direct evidence of the presence of viruses or their components in the organ are available for Human T lymphocytic virus (HTLV)-1, enterovirus, rubella, mumps virus, herpes simplex virus (HSV), Epstein-Barr virus (EBV) and parvovirus in HT.[5] Recently, a few studies have suggested the association of parvovirus infection with thyroiditis.[6]

Parvovirus B19 belongs to the Parvoviridae family of small DNA viruses.[7] Infection with parvovirus B19 (B19) is a global concern and the infection rate is similar in the United States, Europe and Asia, with approximately half of 15-year-old adolescents and over 60% of adults being seropositive.[8,9] Parvovirus B19 is a common human pathogen, which has been linked to autoimmune diseases recently. Both in children and adults, B19 infections are generally considered a cause or trigger of various forms of autoimmune diseases such as rheumatoid arthritis (RA), idiopathic thrombocytopenic purpura (ITP), systemic lupus erythematosus (SLE) and other autoimmune hematologic diseases.[10-16]

Although the association between HT and parvovirus B19 infection is demonstrated, the serological reflection of this association is not clear. The aim of this study was to test for anti-parvovirus IgM and IgG antibodies in adult patients with HT.

MATERIAL AND METHODS

This study was run in the Internal Medicine Department and The Experimental Medical Research Laboratory of İstanbul University in İstanbul, Turkey from April 2010 to March 2011. All patients signed patient consent forms, and the study was conducted in accordance with the principles of the Helsinki Declaration. Ethical approval was obtained from the İstanbul University Ethical Committee. A total of 54 adult patients diagnosed with HT and 35 control subjects were included in the study. HT diagnosis was based on histopathological examination results and increased anti-thyroid antibody titers. Serum thyroid stimulating hormone (TSH), free T3, free T4 and anti-thyroid autoantibodies [anti-thyroid peroxidase antibody (anti-TPO) and anti-thyroglobulin antibodies (Anti-Tg)] were measured on Modular EEE Electrod Elecsys Roche Autoanalyzer (Roche Diagnostics, Basel, Switzerland) using chemiluminescence technique. Histological examination results of thyroid needle aspiration biopsy materials at initial diagnosis were obtained from medical records of the patients (6 months to 25 years earlier). The exclusion criteria were (1) any known current infections, (2) malignant diseases and (3) age ≤18 and ≥65 years.

For detection of anti-parvovirus B19 antibodies, blood samples from all patients and control cases were collected in vacutainer tubes with no anticoagulant. Sample tubes were centrifuged at 3000x g for 10 minutes. Serum samples were transferred to 2-ml Eppendorf microcentrifuge tubes. Samples were stored at -70°C prior to analysis. The sera were tested for the presence of anti-parvovirus B19 IgG and IgM antibodies by enzyme linked immunosorbent assay (ELISA) using the Human Parvovirus B19 (recombinant) ELISA kit (DRG Instruments GmbH, Germany) according to the recommendations of the manufacturer. In brief, antibody specific to parvovirus B19 in the diluted serum (1:100) was expected to bind to recombinant parvovirus B19 antigen, which coated the wells of the microplate. The immune complexes were then detected by antibody to human

anti-human IgG and IgM conjugated with horseradish peroxidase. The enzymatic reaction was stopped by adding the substrate tetramethylebenzidine (TMB) and was read at 450 nm by spectrophotometer. The antibody concentration was calculated as an index value according to the recommendation of the company. The reaction was considered positive when the index value was greater than 1.2 and negative when it was below 0.85. The grey zone was between 0.85-1.2.

STATISTICAL ANALYSES

Baseline information was mostly expressed as mean ± standard deviation (SD) for continuous variables and as frequencies for discredit variables. However, some variables displaying very large differences were expressed as median values. Statistical differences between groups were analyzed with the Mann-Whitney U test for parameters displaying nonparametric distribution or chi-square test for parametric values distributed normally. The statistical significance (*p*-value) was set at <0.05.

RESULTS

Demographic characteristics of the study subjects were shown in Table 1. The mean time from the diagnosis of HT was 5.3±12.0 years. A third (18) of the patients was euthyroid in terms of thyroid functions and not using any substitution, whereas the remaining patients were on thyroxin replacement therapy at varying durations (6 months-25 years).

Anti-parvovirus B19 IgM antibodies were positive in 6 patients (11.1%) and 3 controls (5.7%) while IgG antibodies were present in 14 patients (25.9%) and 8 controls (22.9%) (Figure 1). The difference between the patient and control groups was not significant. There was no relationship between the duration of thyroid disease and the presence of viral antibodies. Any of the B19 M positive patients had a distinctive parvovirus infection history. Patients with HT had no other autoimmune disorder.

DISCUSSION

Although statistically insignificant, the number of patients with antiviral antibodies was higher in the HT group than in the control subjects in this study. The lack of significance may be attributed to the small sample number. Nonetheless, this finding may support the viral contribution in the pathogenesis of HT.

A great amount of evidence suggests that viral and bacterial infections may play a role in the induction of thyroid autoimmunity.[17] Autoimmune thyroiditis has been linked to infections with EBV, HTLV-1, hepatitis C virus, and *Yersinia enteroco-*

TABLE 1: The characteristics of the Hashimoto Thyroiditis and Control Groups.

	Hashimoto thyroiditis (n=54)	Control group (n=35)	p
Age	43.1±12.66	47.03±13.48	0.243
Gender (F/M)	47/7	27/8	0.223
Weight (kg)	66.84±8.17	75.25±12.83	0.288
Height (m)	1.63±0.05	1.52±0.04	0.008
Body mass index (kg/m^2)	30.07±5.86	27.37±3.81	0.245
TSH (0.27-4.2 mIU/L)	median 3.05 (min.0.01,max.78.8)	median 1.4 (min.0.51, max.3.6)	0.018
fT3 (2.63-5.7 pmol/L)	1.14 ±0.26	1.14± 0.19	0.925
fT4 (9.01-18.02 pmol/L)	16.5±7.04	16.09± 2.08	0.777
anti-Tg Ab (10-115 IU/mL)	median 351.1 (min.7, max.1620)	median 7.3 (min. 5, max. 23.5)	0.002
anti-TPO Ab (-<5.6 IU/mL)	median 246 (min.16.8, max.10000)	median 10.6 (min. 10, max. 33.4)	0.002

*mean ± standard deviation

F: Female; M: Male; TSH: Thyroid-stimulating hormone; fT3: Free T3; fT4: Free T4; anti-Tg Ab: Anti-thyroglobulin antibodies; anti-TPO Ab: Anti-thyroid peroxidase antibody; mIU/L: Milliinternational units per liter; pmol/L: Picomole per liter; IU/mL: International units per milliliter; NS: Not significant.

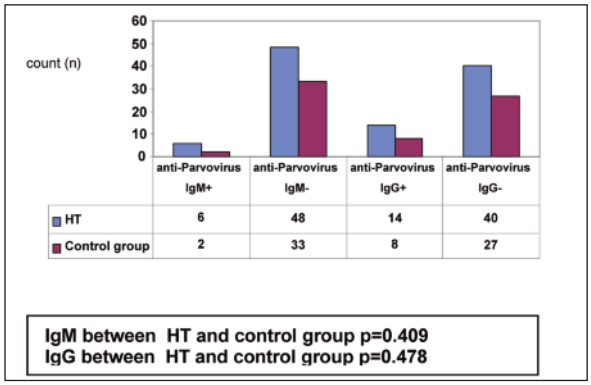

FIGURE 1: Distiribution of Parvovirus B19 in Hashimoto thyroiditis and control groups patients. HT: Hashimoto thyroiditis.
(See for colored form http://tipbilimleri.turkiyeklinikleri.com/)

litica.[5] Specific infections were suggested to be a triggering factor for the initiation of disease by liberating antigens (via cell destruction or apoptosis), by forming altered antigens or causing molecular mimicry, by cytokine and chemokine secretion, and by inducing aberrant HLA-DR expression and Toll-Like Receptor (TLR) activation. TLRs are a family of cell surface receptors that protect mammals from pathogenic organisms such as viruses and are present on non-immune cells including thyrocytes.[18] Moreover, TLR3 recognizes double-stranded (ds) RNA, assumed to be released by viral killing of cells. The dsRNA binding to TLR3, mimicked *in vitro* by incubation with polyinosine-polycytidylic acid [Poly (I:C)], leads not only to the induction of inflammatory responses but also to the development of antigen-specific adaptive immunity. Direct evidence of the presence of viruses or their components in the organ are available for HTLV-1, enterovirus, rubella, mumps virus, HSV, EBV and parvovirus in HT.[5]

There are several publications related to the association between parvovirus and HT. In 1994, Vejlgaard and Nielsen reported a single case of association of HT and human parvovirus infection, and in 2007 Mori and co-workers reported on the intrathyroidal persistence of parvoviral DNA in one patient.[19] Recently, Lehmann et al. presented data on the association of parvovirus B19 infection with the development of HT in children.[6] They analyzed the sera of children with HT for the presence of viral DNA and for antibodies against VP1, VP2, and NS1 proteins. They did not find any difference in terms of antibodies between the patients and controls as in accordance with our study. However, compared to the control group, parvovirus B19 DNA was more frequently detectable in patients; nine patients were shown to produce detectable amounts of virus, whereas only two of the children in the control group were viremic. More recently, Wang et al. showed the presence of B19 nuclear acid and viral protein in HT tissues and suggested that B19 had a potential in the development of adult HT.[20]

As Tozzoli et al. suggest, it is obvious that the presence of antibodies directed towards a virus does not prove that this pathogen is responsible for the disease, especially, when the agent is common in the general population.[4] It is also logical to think that the absence of viral markers at the onset of the disease does not exclude the viral hypothesis, because of the fact that the triggering infection can take place many years ago. It is relevant to look for viral agents in tissues in which they can persist without systemic manifestation.[4] The limitation of this current study is the absence of tissue examination for viral DNA. Furthermore, the small sample size may be another factor for the insignificant results. However, considering that most people are exposed to B19 and the prevalence of antibodies specific for the virus may be approximately 60% in the adult patients, the findings in such a small group like ours may support the above-mentioned cellular studies.[9]

In conclusion, although statistically insignificant, the number of cases with antiviral antibodies was higher in HT patients compared to the control cases in this study. This finding may reflect the importance of viral contribution in the pathogenesis of HT. It is obvious that future studies with larger patient groups may help clarify whether B19 antibodies reflect the contribution of parvovirus infection in HT.

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