

Association between thyroid autoimmunity and *Helicobacter pylori* infection

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Background/Aims: There have been controversial reports linking *Helicobacter pylori* infection to autoimmune thyroid disease (AITD). However, data regarding the relationship are limited for Asian populations, which have an extremely high prevalence of *H. pylori* infection. We performed this study to investigate the association between *H. pylori* infection and AITD in Koreans.

Methods: This study involved adults aged 30 to 70 years who had visited a health promotion center. A total of 5,502 subjects were analysed. Thyroid status was assessed by free thyroxine, thyroid stimulating hormone, and anti-thyroid peroxidase antibody (TPO-Ab). Immunoglobulin G (IgG) antibodies to *H. pylori* were measured as an indication of *H. pylori* infection. We compared the prevalence of TPO-Ab in subjects with and without *H. pylori* infection.

Results: *H. pylori* IgG antibodies were found in 2,875 subjects (52.3%), and TPO-Ab were found in 430 (7.8%). Individuals positive for *H. pylori* Ab were older than those negative for *H. pylori* Ab ($p < 0.01$). The proportion of females was significantly higher in the TPO-Ab positive group (41.0% vs. 64.2%, $p < 0.01$). Prevalence of TPO-Ab positivity was higher in subjects with *H. pylori* infection (8.6% vs. 7.00%, $p = 0.03$), and this association was significant after adjusting for age, sex, and body mass index (odds ratio, 1.02; 95% confidence interval, 1.00 to 1.03; $p = 0.04$).

Conclusions: In our study, prevalence of TPO-Ab positivity is more frequent in subjects with *H. pylori* infection. Our findings suggest *H. pylori* infection may play a role in the development of autoimmune thyroiditis.

Keywords: Thyroid; Autoimmunity; *Helicobacter pylori*

INTRODUCTION

Autoimmune thyroid disease (AITD), one of the most common autoimmune diseases, had several pathogenesis, including both genetic and environmental factors and is one of risk factor for thyroid dysfunction [1,2]. One of potential environmental causes are infectious agents such as *Helicobacter pylori*, which may cause chronic inflammation and autoimmune reactivity in susceptible subjects [3,4]. Although some studies support a role for

H. pylori in AITD, this remains controversial [5-14].

H. pylori infection was prevalent in Asian populations in the last 10 years. Though the prevalence of *H. pylori* infection and annual reinfection rate found to be decreased especially below the age of 40's after introduction of triple therapy, *H. pylori* infection is still prevalent in Korean population [15]. The incidence of *H. pylori* infection in the Korean population above 16 years of age was 59.6% in 2005 [15]. Furthermore, extra-gastric diseases, especially autoimmune diseases that might be

related to *H. pylori* infection have received attention recently [16,17]. However, data regarding the relationship between *H. pylori* infection and thyroid autoimmunity are limited in Asian populations, in which *H. pylori* infection is extremely prevalent.

In the present study we investigated the association between *H. pylori* infection and AITD in the Korean population. Our findings indicated that thyroid autoimmunity is significantly associated with *H. pylori* infection.

METHODS

Subjects

This study was performed in adults aged 30 to 70 years (median, 52) who had visited the health promotion center at Asan Medical Center, Seoul, Korea, from January 2009 to December 2009. Subjects who checked for serum levels of free thyroxine (T₄), thyroid stimulating hormone (TSH), thyroid peroxidase antibody (TPO-Ab) and *H. pylori* immunoglobulin G (IgG) antibodies were included. We excluded subjects with previous histories or family histories of thyroid disease.

Laboratory measurements

Thyroid assessment was done by free T₄, TSH, and TPO-Ab. Serum TPO-Ab concentrations were determined using a BRAHMS anti-TPO radioimmunoassay (RIA) kit (Thermo Scientific, Bonn, Germany) with a functional sensitivity of 30 U/mL. TPO-Ab levels exceeding 60 U/mL were considered positive for TPO-Ab. Levels of serum TSH and free T₄ were measured using a TSH-CTK-3 immunoradiometric assay kit (DiaSorin S.p.A, Saluggia, Italy) and a FT₄ RIA kit (Beckman Coulter, IMMUNOTECH, Prague, Czech Republic), respectively.

Our center uses the Immulite 2000 *H. pylori* IgG system (Siemens AG, Erlangen, Germany) to measure anti-*H. pylori* IgG [18]. This test consists of a solid-phase, 2-step, chemiluminescent enzyme immunoassay.

Definitions

We defined thyroid autoimmunity as positive TPO-Ab, irrespective of the presence of thyroid dysfunction. *H. pylori* infection was defined as positive for IgG antibodies to *H. pylori*. In our center, the results of *H. pylori* IgG

were reported as positive (> 1.5 U/mL), equivocal (0.9 to 1.5 U/mL), and negative (< 0.9 U/mL) [19]. Subjects with equivocal *H. pylori* IgG results were excluded.

Age was stratified by quartile. The quartiles were 30 to 45 years (Q₁), 46 to 51 years (Q₂), 52 to 56 years (Q₃), and 57 to 70 years (Q₄).

Statistical analysis

Continuous variables were expressed as mean ± standard deviation and categorical variables as numbers (percentages). For categorical variables comparisons between groups were performed using the chi-square test and Fisher exact test (two-sided). Multivariate analysis was carried out using a binary logistic regression model. The R software package version 3.0 (R Foundation for Statistical Computing, Vienna, Austria; <http://www.R-project.org>) was used for statistical analysis. All *p* values were two-sided and *p* < 0.05 was considered to denote statistical significance.

RESULTS

A total of 5,967 subjects without past or family histories of thyroid disease were included. Of these, 465 subjects with equivocal results for *H. pylori* IgG antibody were excluded. Finally 5,502 subjects (female 42.8%) were analysed. The mean age was 51.9 years. *H. pylori* infection was found in 2,875 subjects (52.3%), and thyroid autoimmunity was present in 430 (7.8%).

The clinical characteristics of the subjects according to *H. pylori* infection and thyroid autoimmunity are summarized in Tables 1 and 2, respectively. Subjects with *H. pylori* infection were older than those without *H. pylori* infection (*p* < 0.01), and there were significant differences between the age quartiles in the frequency of *H. pylori* infection. The proportions of individuals positive for *H. pylori* Ab were 46.1%, 56.1%, 52.0%, and 53.5%, in Q₁, Q₂, Q₃, and Q₄, respectively (*p* < 0.01) (Table 1). On the other hand, the proportion of females was significantly higher in the TPO-Ab positive group than in the TPO-Ab negative group (64.2% vs. 41.0%, *p* < 0.01) (Table 2).

We compared thyroid autoimmunity according to *H. pylori* infection. Prevalence of TPO-Ab positivity was higher in subjects with *H. pylori* infection (246 out of 2,875 subjects [8.6%] vs. 184 out of 2,627 subjects [7.0%],

Table 1. Characteristics of the subjects according to *Helicobacter pylori* immunoglobulin G antibody status

Characteristic	Total (n = 5,502)	<i>H. pylori</i> Ab (-) (n = 2,627)	<i>H. pylori</i> Ab (+) (n = 2,875)	p value
Age, yr	51.9 ± 8.3	51.4 ± 8.6	52.2 ± 8.0	< 0.01
Age quartile ^a				< 0.01
Q1	1,183	637 (53.8)	546 (46.1)	
Q2	1,477	649 (43.9)	828 (56.1)	
Q3	1,268	609 (48.0)	659 (52.0)	
Q4	1,574	732 (46.5)	842 (53.5)	
Female sex	2,353 (42.8)	1,131 (43.1)	1,653 (57.5)	0.70
TSH, mU/L ^b	2.2 ± 2.1	2.1 ± 2.1	2.2 ± 2.0	0.08
Free T ₄ , ng/dL	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	0.06

Values are presented as mean ± SD or number (%).

Ab, antibody; TSH, thyroid stimulating hormone; T₄, thyroxine.

^aAge was stratified by quartile. The quartiles were 30 to 45 (Q1), 46 to 51 (Q2), 52 to 56 (Q3), and 57 to 70 (Q4).

^bGeometric mean.

Table 2. Characteristics of subjects according to TPO-Ab status

Characteristic	Total (n = 5,502)	TPO-Ab (-) (n = 5,072)	TPO-Ab (+) (n = 430)	p value
Age, yr	51.9 ± 8.3	51.8 ± 8.3	52.6 ± 8.1	0.05
Age quartile ^a				0.34
Q1	1,183	1,094 (92.3)	89 (7.5)	
Q2	1,477	1,375 (93.1)	102 (6.9)	
Q3	1,268	1,164 (91.8)	104 (8.2)	
Q4	1,574	1,439 (91.4)	135 (8.6)	
Female sex	2,353 (42.8)	2,077 (41.0)	276 (64.2)	< 0.01
TSH, mU/L ^b	2.2 ± 2.1	2.1 ± 2.0	2.7 ± 3.0	< 0.01
Free T ₄ , ng/dL	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.3	0.29

Values are presented as mean ± SD or number (%).

TPO-Ab, thyroid peroxidase antibody; TSH, thyroid stimulating hormone; T₄, thyroxine.

^aAge was stratified by quartile. The quartiles were 30 to 45 (Q1), 46 to 51 (Q2), 52 to 56 (Q3), and 57 to 70 (Q4).

^bGeometric mean.

$p = 0.03$) (Fig. 1). We performed a multivariate analysis to adjust for the possible interaction between thyroid autoimmunity and various clinical parameters. The serologic results of *H. pylori* were significantly related to TPO-Ab positivity after adjusting for age, sex, and body mass index (odds ratio, 1.02; 95% confidence interval, 1.00 to 1.03; $p = 0.04$).

DISCUSSION

Infectious agents have been implicated in the pathogenesis of autoimmunity [20]. Epidemiological and serolog-

ical surveys have suggested the possibility of a link between *Yersinia enterocolitica* infection and Graves disease, a link which might be explained by molecular mimicry [21,22]. Infectious agents may lead to thyroid autoimmunity by a variety of mechanisms, such as inducing modification of self-antigens, mimicry of self-molecules, activation of polyclonal T cells, alteration of the idiotype network, formation of immune complexes, and induction of major histocompatibility complex molecules on thyroid epithelial cells [4]. A homologous 11-residue peptide in both gastric parietal cell antigen and thyroid peroxidase suggests the existence of an epitope common to both of the two antigens [23]. There may be cross

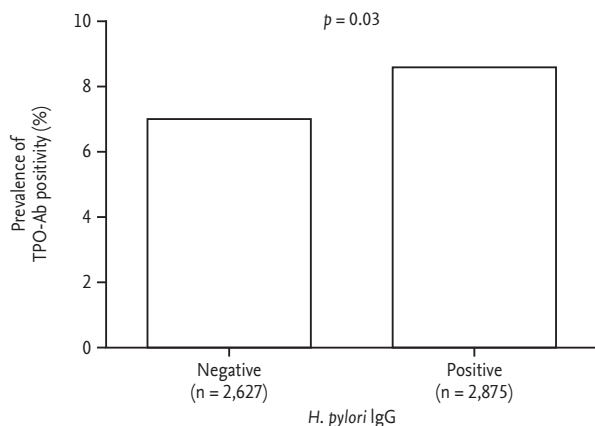


Figure 1. Proportion of individuals positive for anti-thyroid peroxidase antibody (TPO-Ab) according to *Helicobacter pylori* infection status. IgG, immunoglobulin G.

reaction between antibodies produced during *H. pylori* infection and thyroid antigens, leading to development of AITD. Interestingly, elevated chemokine response to *H. pylori* have been observed in AITD patients [24].

H. pylori is a spiral-shaped, flagellated, gram-negative bacterium, and colonizes the stomach of about 50% of the world's population [16]. It can cause dyspepsia, acute and chronic gastritis, peptic ulceration, mucosa associated lymphoid tissue lymphoma, and gastric adenocarcinoma. In particular, the most virulent strain of *H. pylori* was identified by the presence of cytotoxin-associated gene A (CagA), and individuals infected with the CagA-positive strain of *H. pylori* have an increased risk of peptic ulcer and gastric cancer [25]. Furthermore, several studies have reported a significant correlation between CagA-positive strains and AITD. Because our study was based on data from health check-ups, CagA status was not investigated.

Previous studies regarding the association between *H. pylori* and AITD have generally been from Italy, Czechoslovakia, and Iran, in which *H. pylori* infections are prevalent. In Korea, the seroprevalence of *H. pylori* infection was 59.6% in 2005 and decreased to 54.4% in 2011, according to Lim et al. [26]. It is unclear whether the prevalence of *H. pylori* infection and a decreasing trend of *H. pylori* seroprevalence will affect the association between *H. pylori* and AITD.

Our study had several limitations. First, we defined *H. pylori* infection based on a serologic test, which detects

both past and current infections. Second, our definition of AITD did not differentiate between Graves disease and Hashimoto thyroiditis. Third, we did not test for the presence of CagA because our study was based on health check-up data. Finally, there might be concern about false positive results in *H. pylori* IgG test and TPO-Ab assay. However, *H. pylori* IgG test is highly specific and had no cross-reactivity to *Campylobacter coli* microorganism. We also excluded patients with equivocal results to avoid a concern of false positive results. TPO-Ab assay has cross-reactivity with human anti-Tg antibodies and human Tg. However, anti-Tg antibody could one of other representative of thyroid autoimmunity and we excluded subjects with history of thyroid disease. Therefore, there might be little concern about false positive results.

In conclusion, our findings suggest that *H. pylori* infection may be associated with AITD. Further studies are needed to confirm the role of *H. pylori* infection in AITD.

KEY MESSAGE

1. Infectious agents have been implicated in the pathogenesis of autoimmunity.
2. In our study, prevalence of thyroid peroxidase antibody positivity is more frequent in subjects with *Helicobacter pylori* infection.
3. *H. pylori* infection may be associated with autoimmune thyroid disease.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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