Autoimmune thyroiditis and *Helicobacter pylori* – is there a connection?

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Abstract

OBJECTIVES: In this study we examined the anti-*Helicobacter pylori* (anti-H. pylori) antibodies in patients with autoimmune thyroiditis, with and without different polyglandular involvement, and in healthy controls.

MATERIAL & METHODS: Patients with autoimmune thyroiditis (AT) were divided into three groups: Group A: 23 patients with isolated AT, Group B: 30 patients with AT as a part of polyglandular activation of autoimmunity, and Group C: 7 patients with AT as a part of autoimmune polyglandular syndrome type II. Thirty healthy individuals served as controls (Group D). Anti-H. pylori antibodies were determined first by ELISA for classes IgG, IgA, and IgM, and subsequently by immunoblot for classes IgG and IgA.

RESULTS: ELISA: The number of patients with IgA antibodies in Group A (39%) and Group B (30%) differed significantly from controls (7%, $p<0.05$).
IMMUNOBLOT: Anti-CagA antibodies were found in 13% of patients in Group A, 7% of Group B, 0% of Group C, and 20% of Group D. A higher seroprevalence, as compared to controls, was found for IgG to the VacA ($p=0.01$), 30 kDa ($p=0.001$), and 17 kDa ($p=0.008$) antigens in Group A and for IgG to the 30 kDa antigen in Group C ($p=0.037$). A significantly higher seroprevalence, as compared to controls, was likewise found for IgA to the 17 kDa antigen in Group A ($p=0.015$).

CONCLUSIONS: A different distribution of antibodies to H. pylori antigens was found in patients with isolated AT compared to patients with AT coupled with a polyglandular syndrome.

ORIGINAL ARTICLE

Abbreviations & Units

21-OH:	21-hydroxylase
APS:	autoimmune polyglandular syndrome
APSII:	autoimmune polyglandular syndrome type II
AT:	autoimmune thyroiditis
DM:	diabetes mellitus
ELISA	enzyme-linked immunosorbent assay
H-pylori	*Helicobacter pylori*
HLA:	human leukocyte antigen
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	index of positivity
kDa	kiloDalton
PAA:	polyglandular autoimmunity activation
RIA	radioimmuno assay
Tg:	thyroglobulin
TPO:	thyroid peroxidase

Introduction

Autoimmune thyroiditis (AT) is one of the most frequently encountered endocrinopathies belonging to organ-specific autoimmune diseases. AT can also occur as a part of autoimmune polyglandular syndrome (APS) or of polyglandular autoimmunity activation (PAA). APS is classified into three types: APS type I, APS type II (APSII) and APS type III [17]. PAA is a term for a group of autoimmune endocrinopathies including AT or diabetes mellitus (DM) type I where an associated occurrence of autoantibodies against other endocrine organs (without functional impairment leading to clinical manifestation) can be found [12]. In female patients with autoimmune disorders of the thyroid gland, the most frequently encountered finding is simultaneous occurrence of antibodies against so-called steroid-producing cells in the ovary in combination with antibodies against the upper adrenal layers [27]. Similar to autoimmune thyropathies, this disorder has multifactorial and polygenetic origin, with involvement of both extrinsic and intrinsic etiopathogenic mechanisms. Intrinsic factors include genetic predisposition involving human leukocyte antigens (HLA), Fas/FasL, cytotoxic T lymphocyte-associated protein 4, and other antigens [28]. External factors of interest are infectious agents, e.g. *Yersinia enterocolitica* [3, 4]. Recently, several authors described linkage of thyroid disease to infection by *Helicobacter pylori* [6, 9].

H. pylori is a gram-negative microaerophile bacteria causing chronic, usually lifetime infection. It is manifested by gastritis that can progress into ulceration of the stomach and duodenum, gastric adenocarcinoma, or mucosa-associated lymphoid tissue lymphoma [8]. The pathogenicity is linked to the production of various proteins either released by H. pylori or bound to its cell membrane [13, 14]. One of these virulence factors is CagA protein. A recent meta-analysis of case-control studies indicates that infection with CagA-positive strains leads to progression of more serious diseases [11]. H. pylori infection has also been linked to many extragastroduodenal diseases: cardiovascular diseases, respiratory tract diseases, growth retardation, cerebrovascular diseases, DM, headache and migraine, Raynaud's syndrome, and last but not least to autoimmune diseases [29].

In patients with autoimmune thyropathies, particularly with the atrophic form of AT, as well as in patients with Graves' thyrotoxicosis and Hashimoto's thyroiditis, an increased prevalence of H. pylori has been found [6, 9]. This finding is supported by elevated levels of anti-H. pylori IgG antibodies and by breath test results. In patients suffering from AT and infection with H. pylori, abnormalities in the secreting function of the stomach were found [6]. However, other authors have failed to find a direct involvement of H. pylori infection in the etiology of AT [27].

Bertalot and coworkers reported a decrease in anti-thyroid autoantibodies after eradication of H. pylori infection [5]. Reduction of anti-thyroid peroxidase (TPO) and anti-thyroglobulin (Tg) autoantibodies after H. pylori eradication emphasizes the necessity of examining patients with AT for the presence of H. pylori, due to the clinically beneficial effect of such eradication.

In this study we examined three groups of AT patients with or without polyglandular involvement for the presence of anti-H. pylori antibodies and distribution of reactivity to individual H. pylori antigens.

Material & Methods

Patients

Participants in the study were selected from patients of the Institute of Endocrinology, Prague, Czech Republic, and of 3rd Clinic of Internal Medicine, 1st Medical Faculty, Charles University, Prague.

Based on detection of organ-specific autoantibodies and on the clinical state, the patients were divided into three groups:

Group A:	23 patients with isolated AT
Group B:	30 AT patients with PAA
Group C:	7 AT patients with APSII (Addison's disease and/or DM type I)
Group D:	30 healthy individuals without autoimmune endocrinopathy

The mean age (standard deviation) of patients in Group A was 49.2 (14.7) years, in Group B 46.9 (16.3) years, in Group C 45.9 (15.6) years, and in Group D 33.9 (10.7) years. Group A consisted of 21 women and 2 men, Group B of 29 women and 1 man, Group C of 5 women and 2 men, and Group D of 16 women and 14 men.

The diagnosis of AT in Groups A–C was based on clinical and ultrasound findings and positivity for antibodies against TPO and/or Tg. Patients had been monitored for more than 10 years.

Group B included patients with AT with seroprevalence for other organ-specific autoantibodies. Patients of Group C were selected on the basis of anamnestic data regarding adrenocortical insufficiency, positivity for autoantibodies against 21-hydroxylase (21-OH), and presence of AT and/or DM type I based on clinical find-

ings. All patients were on corticosteroid replacement therapy during the study and were euthyroid at the time of the study.

Organ-specific autoantibodies

Autoantibodies against TPO and Tg were detected in sera by ELISA (kit Autostat II, Cogent Diagnostics Ltd, Penicuik, UK).

Autoantibodies against antigens of adrenals, ovary, and islet cells were determined by indirect immunofluorescence on monkey tissue (Binding Site, Birmingham, UK). Briefly, the sections were incubated with patient sera for 30 min and, after thorough washing, were incubated with fluorescein-conjugated antibody against human immunoglobulin (Binding Site). The evaluation was performed with a fluorescence microscope at 490 nm.

Autoantibodies against glutamic acid decarboxylase, specific islet antigen 2, and 21-OH were detected by RIA (Cis Bio International, Gif sur Yvette, France).

Serological determination

Quantitative determination of antibodies against H. pylori in sera was carried out for IgG, IgA, and IgM by commercial kits (EIA-H. pylori, Test-Line, Brno, Czech Republic). The results were expressed by indices of positivity (IP = ratio of the mean absorbance of the sera and cut-off) and evaluated as positive when IP > 1.2.

Quantitative determination of IgG antibodies against CagA protein of H. pylori was carried out with a commercial kit (H. pylori 120 [CagA], ELISA Test-Line) and the results were expressed by IP with positive defined as IP > 1.2.

Detection of IgG and IgA antibodies to specific H. pylori antigens (120 kDa, 87 kDa, 33 kDa, 30 kDa, 29 kDa, 26 kDa, 19 kDa, and 17 kDa) was carried out by commercial kits (BLOT H. pylori IgG and IgA, Test-Line). Sera reacting with one of the antigens 120 kDa (CagA), 87 kDa (VacA), or 33 kDa, or with at least two of the antigens 30 kDa, 29 kDa, 26 kDa, 19 kDa, and 17 kDa, were evaluated as positive.

Statistics

Statistical evaluation was carried out by two-sided Fisher's exact test using the statistical software R (R Development Core Team, Vienna) [22].

Results

Anti-H. pylori antibodies were detected in all three Groups of patients and in controls first by ELISA for IgG, IgA, and IgM, and for IgG to CagA and subsequently by immunoblot for IgG and IgA to specific H. pylori antigens.

Anti-H. pylori IgG antibodies were found in 26% patients with isolated AT (Group A), in 17% of AT patients with PAA (Group B), in 29% of AT patients with APSII (Group C), and in 13% of healthy controls (Group D). Anti-H. pylori IgA antibodies were found in 39% of Group A, in 30% of Group B, in 29% of Group C, and in 7% of Group D. Anti-H. pylori IgM antibodies were found in 17% of Group A, in 17% of Group B, in 29% of Group C, and in 17% of Group D. While the prevalence of anti-H. pylori IgA and IgG antibodies was higher for all three groups compared to controls, the difference was significant only for IgA in Groups A and B ($p<0.05$) (Fig. 1).

Anti-CagA IgG antibodies were found in 13% of Group A, in 7% of Group B, in 0% of Group C, and in 20% of Group D (data not shown). The results are not statistically significant due to the small number of persons in individual groups, especially in Group C (n = 7).

Qualitative determination of antibodies by immunoblot has shown that compared with healthy controls,

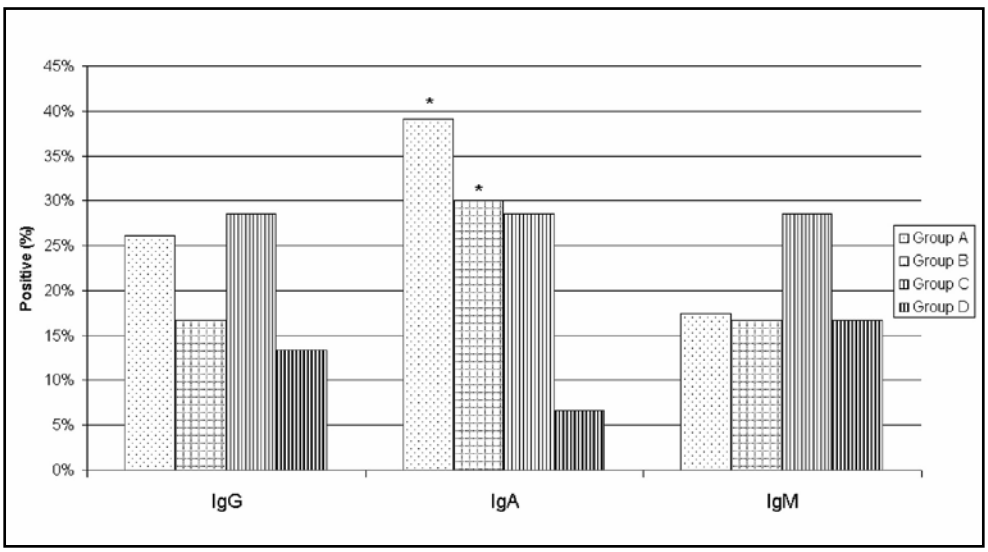

Figure 1. IgG, IgA, and IgM antibodies to H. pylori as determined by ELISA. * denotes statistically significant differences, $p<0.05$

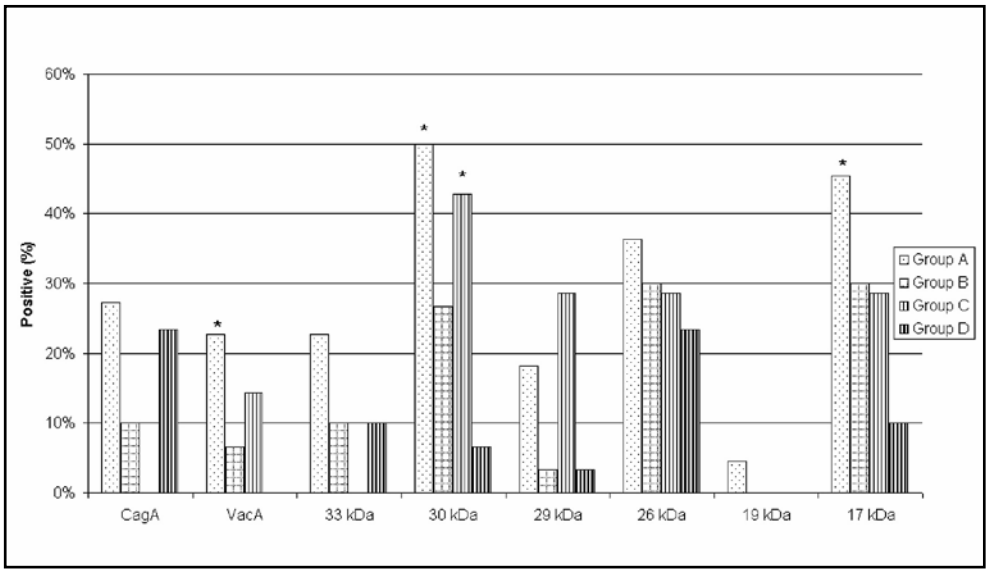

Figure 2. IgG antibodies to different H. pylori antigens as determined by immunoblot.
* denotes statistically significant differences, p<0.05

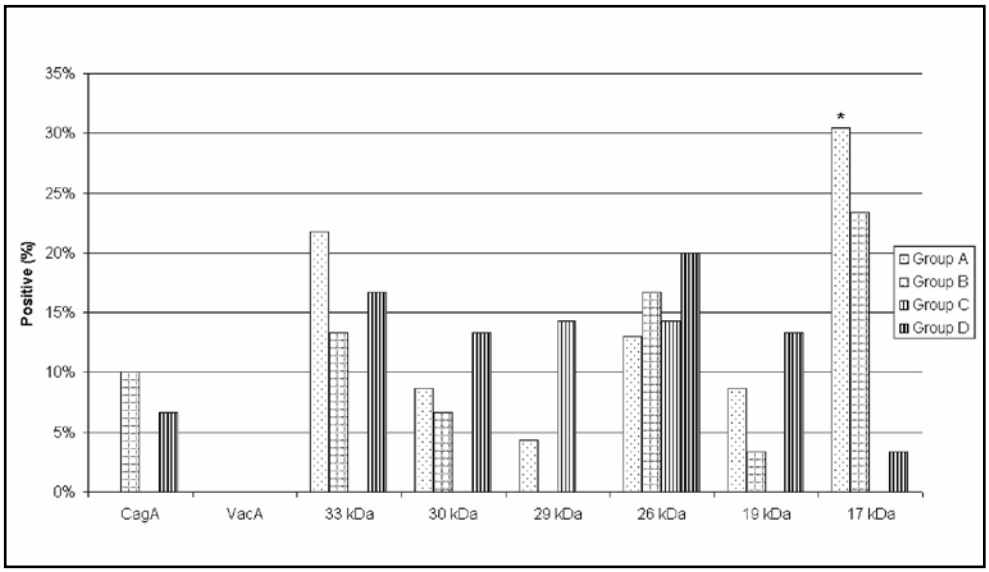

Figure 3. IgA antibodies to different H. pylori antigens as determined by immunoblot.
* denotes statistically significant differences, p<0.05

IgG antibodies were more frequently found to VacA (p=0.01), 30kDa (p=0.001) and 17 kDa (p=0.008) in Group A and to 30 kDa in Group C (p=0.037) (Fig. 2).

Finally, compared to Group D, IgA antibodies were significantly more frequently found to 17 kDa in patients with isolated AT (Group A) as compared to controls (p=0.015) (Fig. 3).

Discussion

The results of this study show the connection of H. pylori seropositivity with AT in all three groups of patients studied. The seroprevalence was most significant for IgA in Group A (patients with isolated AT) and Group B (AT patients with PAA). Similar results were published previously [6, 9, 23].

In our earlier studies, patients with polyglandular involvement (APSII and PAA) demonstrated only minimal genetic variation. and therefore the different clinical development might be due to epigenetic factors. In contrast, there was a difference in HLA antigen expression between patients with isolated AT and both groups with polyglandular involvement (PAA and APSII) [10]. In addition, we demonstrated a relationship between AT and hypersensitivity to heavy metals [2, 21, 24, 25, 26, 30] relative to genetic background [20].

Infection with H. pylori in connection to AT has been studied by many researchers [5, 6, 9, 23]. The putative mechanism to explain how H. pylori infection in the stomach can pathogenically influence remote organs is the induction of an autoimmune reaction by molecular mimicry [16, 18]. Antigens involved in this cross-reaction were partially identified as Lewis antigens of blood groups [1, 15]. In addition, eradication of H. pylori infection reduced the symptoms of autoimmune process,

i.e., caused a decrease in the levels of anti-thyroid autoantibodies [5]. Several authors described a relationship between H. pylori infection and gastric autoimunity [7, 19].

In contrast to another study [9], we could not show a significantly higher prevalence of antibodies to CagA in patients with AT. However, we found a higher prevalence of such antibodies in patients with isolated AT (Group A) as compared to patients with polyglandular involvement. Furthermore, we demonstrated the significantly increased prevalence of antibodies to low molecular weight antigens (17 kDa and 30 kDa) in AT patients.

The different findings of seropositivity to H. pylori antigens in all groups with AT suggest that the H. pylori infection may activate AT. On the other hand, activation might be mediated by different H. pylori antigens in the groups with the different occurrence of AT.

In conclusion, AT may not necessarily represent a totally independent nosological entity but rather a manifestation of various clinical syndromes with different genetic and extraneous factors. The results of this study support a hypothesis of different epi-immunogenetic backgrounds of AT either as an isolated disorder or as a part of polyglandular autoimmune disease.

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