Anti-\textit{Helicobacter Pylori}, Anti-Thyroid Peroxidase, Anti-Thyroglobulin and Anti-Gastric Parietal Cells Antibodies in Czech Population

I. ŠTERZL\textsuperscript{1,2}, P. HRDÁ\textsuperscript{1,2}, P. MATUCHA\textsuperscript{1}, J. ČEŘOVSKÁ\textsuperscript{1}, V. ZAMRAZIL\textsuperscript{1}

\textsuperscript{1}Institute of Endocrinology, Prague and \textsuperscript{2}Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University and General Faculty Hospital, Prague, Czech Republic

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Summary

Autoimmune thyropathies are frequently linked to many infections, such as \textit{Helicobacter pylori}, which are also supposed to play a role in their pathogenesis. The aim of this study was to evaluate the relationships between thyroid and gastric autoimmunity and \textit{H. pylori} infection on a large sample of Czech population (n=1621) by monitoring the autoantibodies against thyroglobulin (anti-Tg) and thyroid peroxidase (anti-TPO) and gastric parietal cell (anti-GPC, representing thyrogastric syndrome) in correlation with antibodies against \textit{Helicobacter pylori} (anti-\textit{H. pylori}) of classes IgG and IgA. The interrelation between autoantibodies and \textit{H. pylori} antibodies was assessed by \textit{H. pylori} seropositivity. In \textit{H. pylori} seropositive persons as compared to seronegative irrespective of age and sex, a higher occurrence of anti-TPO (10.4 % vs. 5.8 %, \textit{p}=0.001) and anti-GPC (6.1 % vs. 1.7 %, \textit{p}<0.001) was found. Differences in anti-TPO occurrence were significant in both men (7.0 % vs. 3.3 %, \textit{p}=0.03) and women (12.7 % vs. 8.0 %, \textit{p}=0.02), differences in anti-GPC occurrence were significant only in women (7.2 % vs. 1.7 %, \textit{p}<0.001). Results of this study support the idea of a connection between infection of \textit{H. pylori} and the occurrence of anti-TPO autoantibodies representing thyroid autoimmunity and gastric parietal cells autoantibodies representing the thyrogastric syndrome.

Key words

\textit{Helicobacter pylori} • Thyroid peroxidase • Thyroglobulin • Gastric parietal cells • Antibodies

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). (Muir et al. 1995, Laureti et al. 1998). As serological markers of AT serve autoantibodies against thyroid peroxidase (anti-TPO) and against thyroglobulin (anti-Tg). Their occurrence has been reported in healthy women in the range from 9 % to 26 % and in healthy men in the range from 3 % to 12 % (Prentice et al. 1990, Vanderpump et al. 1995, Hawkins et al. 1980).

The etiological causes for autoimmune thyroiditis development are multifactorial, involving genetic predisposition (Hrdá et al. 2004, Todd et al. 1988) and external factors, most common being infections, such as \textit{Yersinia enterocolitica} (Bech et al. 1977, Bech et al. 1974) and \textit{Helicobacter pylori} (de Luis et al. 1998, Figura et al. 1999). \textit{H. pylori} causes chronic, usually lifetime infection and is associated with a wide spectrum of other clinical diseases, ranging from peptic ulcer disease to gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (Suerbaum and Michetti 2002) and also with many extragastrointestinal diseases: cardiovascular diseases, respiratory tract diseases, growth retardation, cerebrovascular diseases, headache and migraine, Raynaud’s syndrome and with organ specific autoimmune diseases (autoimmune thyropathies, diabetes mellitus), systemic autoimmune diseases (Tsang and Lam 1999, Figura et al. 1999). In patients with autoimmune
thyropathies an increased prevalence of *H. pylori* has been found (de Luis *et al.* 1998, Figura *et al.* 1999). Bertalot *et al.* (2004) reported a decrease in anti-thyroid autoantibodies after eradication of *H. pylori* infection.

Reports about *H. pylori* infection indicate higher infection rates (about 90 % during adulthood) in developing countries (Bardhan 1997), while infection rates in developed countries were about 60 % in higher age groups (Frenck and Clemens 2003). The World Health Organization estimates show that approximately 50 % of the world’s populations are infected with *H. pylori*, however, the majority of infected subjects develop no clinical symptoms (Rothenbacher and Brenner 2003). The occurrence of antibodies against gastroparietal cells of the stomach has been reported as high as 12 % (Šterzl *et al.* 1996).

Autoimmune gastritis and its sequel gastric atrophy predispose the subject to, albeit weakly, development of adenocarcinoma in the stomach, however, a strong association with infectious gastritis was recognized well before the identification of *H. pylori* as the cause of gastritis in this context.

As the most readily demonstrable serologic marker of autoimmune gastritis serve the gastric parietal cell autoantibodies, which, in diagnostic laboratories, are usually detected by immunofluorescence on a frozen section of murine stomach. They are highly associated with histologic evidence of gastritis and particularly with fundal gastritis (Whittingham and Mackay 2002).

The aim of this study was to evaluate the relationships between thyroid and gastric autoimmunity and *H. pylori* infection on a large sample of Czech population by monitoring the autoantibodies against thyroglobulin (anti-Tg) and thyroid peroxidase (anti-TPO) and gastric parietal cell (anti-GPC, representing thyrogastriic syndrome) in correlation with antibodies against *Helicobacter pylori* (anti-*H. pylori*) of classes IgG and IgA.

**Methods**

Subjects were selected from the population of the regions of Jablonec nad Nisou (Zamrazil *et al.* 2004), Příbram and Ždár nad Sázavou in the years 2004-2006. (n=1621, mean age ± SD: 27.7±18.7; men: n=700, 25.2±18.8 years, women: n=921, 29.5±18.4 years). The ethical prerequisite of informed consent of the people involved was met.

Antibodies against *H. pylori* IgG and *H. pylori* IgA were determined using ELISA kits Pyloriset EIA-G III Pyloriset EIA-A III from Orion Diagnostica. Results were considered positive when higher than 20 U/ml. Anti-Tg and anti-TPO autoantibodies were determined using ELISA kits AESKULISA a-Tg (results were considered borderline when higher than 125 IU/ml and positive when higher than 150 IU/ml) and AESKULISA a-TPO (results were considered borderline when higher than 25 IU/ml and positive when higher than 50 IU/ml) (AESKU.DIAGNOSTICS, Wendelsheim, Germany). Autoantibodies against gastric parietal cells were determined using the indirect fluorescence kit Rat Kidney, Stomach IFA Kit from The Binding Site Limited (Birmingham, UK).

People were considered positive to *Helicobacter pylori* when having positive at least one antibody. People were considered as showing signs of thyroid autoimmunity, when positive at least to one of the anti-Tg and anti-TPO autoantibodies.

Differences in the prevalences were evaluated using two-sided Fisher’s exact test using R statistical package (R Development Core Team 2007).

**Results**

**Occurrence of antibodies and autoantibodies**

The occurrence of anti-*H. pylori* IgG was 35.6 %, anti-*H. pylori* IgA 34.2 %, anti-TPO 8.0 %, anti-Tg 4.8 % and anti-GPC 3.8 % (Fig. 1). Women showed higher occurrence of anti-*H. pylori* IgG, anti-Tg and anti-TPO and lower occurrence of anti-GPC than men (Fig. 1). Persons older than 18 years showed higher occurrence of anti-*H. pylori* and of all monitored
autoantibodies (Fig. 2, p<0.001). The age dependence of anti-
*H. pylori* and of all monitored autoantibodies is shown in Figure 3.

**Relationship of the occurrence of autoantibodies on *H. pylori* seropositivity**

In *H. pylori* seropositive persons as compared to seronegative irrespective of age and sex higher occurrence of anti-TPO (10.4 % vs. 5.8 %, p=0.001) and anti-GPC (6.1 % vs. 1.7 %, p<0.001) was found. Differences in anti-TPO occurrence were significant in men (7.0 % vs. 3.3 %, p=0.03) and also in women (12.7 % vs. 8.0 %, p=0.02), anti-GPC occurrence only in women (7.2 % vs. 1.7 %, p=0.001). In persons up to 18 years of age, irrespective of sex, a difference showed up in anti-TPO (4.7 % vs. 2.1 %, p=0.05) and anti-GPC occurrence (3.9 % vs. 0.9 %, p=0.004), with respect to sex only in young females a difference in the anti-GPC occurrence (4.6 % vs. 0.7 %, p=0.02) appeared. In persons older than 18 years, irrespective of sex, a difference in anti-GPC occurrence (7.2 % vs. 3.5 %, p=0.04) appeared, with respect to sex however, only in women (8.3 % vs. 3.2 %, p=0.02) (Table 1).

**Relationship of anti-GPC and anti- *H. pylori* occurrence on the positivity of anti-thyroid autoantibodies**

In persons with positive autoantibodies against any of the thyroid antigens higher anti-*H. pylori* IgG (44.4 % vs. 34.5 %, p=0.01) and IgA (43.9 % vs. 33.0 %, p=0.005) and anti-GPC (7.2 % vs. 3.3 %, p=0.02) were found as compared to autoantibody negative persons irrespective of age and sex. With respect to sex differences were found for anti-*H. pylori* IgA in women (44.4 % vs. 34.3 %, p=0.03) and for anti-GPC in men (8.5 % vs. 2.5 %, p=0.04). These differences were not confirmed in the age group under 18 years, in the group older than 18 years only the difference for anti-GPC in men was confirmed (Table 2).

**Discussion**

Infection with *H. pylori* in connection to autoimmune thyroiditis has been studied by many researchers (Bertalot et al. 2004, de Luis et al. 1998, Figura et al. 1999, Raymond et al. 2000). 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 (Moran et al. 1996, Negrini et al. 1996). Antigens involved in this cross-reaction were partially identified as Lewis antigens of blood groups (Appelmelk et al. 1996, Moran 1996). 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 (Bertalot et al. 2004). Several authors described a relationship between H. pylori infection and gastric autoimmunity (D'Elios et al. 2004, Presotto et al. 2003).

Detection of antibodies against CagA antigen of H. pylori has not been subject of this study, however, in a previous study (Šterzl et al. 2006), we could not show a significantly higher prevalence of antibodies to CagA in patients with AT as compared with controls, in contrast to another study (Figura et al. 1999).

Our study of 1621 people is, up to now, the largest study of H. pylori seropositivity reported in the Czech Republic. The population sample was selected from different regions of the Czech Republic during 2004-2006. In our study, the prevalence is somewhat higher than in other developed countries (anti-H. pylori IgG 35.6 %, IgA 34.2 %.) (Goodman and Correa 1995, Pounder and Ng 1995). Bureš et al. (2006) showed similar results in the Czech Republic in spite of using a different method, namely the urease breath test. Roberts et al. (2000) showed that the sensitivity and specificity of serological tests is generally lower than those of breath tests. In accordance with other authors (Sorberg et al. 2003, Robertson et al. 2003) we found an age dependence of the occurrence of H. pylori seropositivity increasing with age. The observed occurrence of anti-TPO (8.0 %) and anti-Tg (4.8 %) in the population under study, without regard to age or gender was slightly lower, as compared with other authors (Tajtaková et al. 2000, Prummel and Wiersinga 2005, Vanderpump et al. 1995).

In accordance with other authors (Vanderpump et al. 1995, Hawkins et al. 1980, Prentice et al. 1990) the occurrence of anti-Tg and anti-TPO autoantibodies increased with age – anti-Tg (6.8 % in older vs. 2.9 % in younger, p<0.001), anti-TPO (13.3 % vs. 2.9 %, p<0.001) and certain results were more frequent in women,

**Table 1.** Significant differences between H. pylori negative and H. pylori positive persons.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Gender</th>
<th>Antibody</th>
<th>H. pylori positive</th>
<th>H. pylori negative</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>All</td>
<td>anti-TPO</td>
<td>10.4 %</td>
<td>5.8 %</td>
<td>0.001</td>
</tr>
<tr>
<td>All</td>
<td>All</td>
<td>anti-GPC</td>
<td>6.1 %</td>
<td>1.7 %</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All</td>
<td>M</td>
<td>anti-TPO</td>
<td>7.0 %</td>
<td>3.3 %</td>
<td>0.032</td>
</tr>
<tr>
<td>All</td>
<td>F</td>
<td>anti-TPO</td>
<td>12.7 %</td>
<td>8.0 %</td>
<td>0.022</td>
</tr>
<tr>
<td>All</td>
<td>F</td>
<td>anti-GPC</td>
<td>7.2 %</td>
<td>1.7 %</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Children and adolescents</td>
<td>All</td>
<td>anti-TPO</td>
<td>4.7 %</td>
<td>2.1 %</td>
<td>0.046</td>
</tr>
<tr>
<td>Children and adolescents</td>
<td>All</td>
<td>anti-GPC</td>
<td>3.9 %</td>
<td>0.9 %</td>
<td>0.004</td>
</tr>
<tr>
<td>Children and adolescents</td>
<td>F</td>
<td>anti-GPC</td>
<td>4.6 %</td>
<td>0.7 %</td>
<td>0.015</td>
</tr>
<tr>
<td>Adults</td>
<td>All</td>
<td>anti-GPC</td>
<td>7.2 %</td>
<td>3.5 %</td>
<td>0.039</td>
</tr>
<tr>
<td>Adults</td>
<td>F</td>
<td>anti-GPC</td>
<td>8.3 %</td>
<td>3.2 %</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Children and adolescents - persons under 18 years of age. Adults – persons older than 18 years.

**Table 2.** Significant differences between anti-thyroid autoantibody negative and anti-thyroid autoantibody positive persons.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Gender</th>
<th>Antibody</th>
<th>Thyroid ab* positive</th>
<th>Thyroid ab* negative</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>All</td>
<td>anti-H. pylori IgG</td>
<td>44.4 %</td>
<td>34.5 %</td>
<td>0.010</td>
</tr>
<tr>
<td>All</td>
<td>All</td>
<td>anti-H. pylori IgA</td>
<td>43.9 %</td>
<td>33.0 %</td>
<td>0.005</td>
</tr>
<tr>
<td>All</td>
<td>All</td>
<td>anti-GPC</td>
<td>7.2 %</td>
<td>3.3 %</td>
<td>0.019</td>
</tr>
<tr>
<td>All</td>
<td>F</td>
<td>anti-H. pylori IgA</td>
<td>44.4 %</td>
<td>34.3 %</td>
<td>0.031</td>
</tr>
<tr>
<td>All</td>
<td>M</td>
<td>anti-GPC</td>
<td>8.5 %</td>
<td>2.5 %</td>
<td>0.039</td>
</tr>
<tr>
<td>Adults</td>
<td>M</td>
<td>anti-GPC</td>
<td>12.9 %</td>
<td>3.7 %</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Children and adolescents - persons under 18 years of age. Adults – persons older than 18 years. *autoantibody.
specifically anti-Tg (6.6 % vs. 2.4 %, p<0.001), and anti-TPO (10.3 % vs. 4.9 %, p<0.001).

Several authors point to the relationship of autoimmune thyroiditis and Helicobacter pylori infection (de Luis et al. 1998, Figura et al. 1999). Helicobacter pylori might be the possible etiological factor for autoimmune thyroiditis development. A significant interaction between HLA-DRB1*0301 and H. pylori infection was present in AT patients and not controls (Larizza et al. 2006).

In the group of the H. pylori seropositive subjects, higher occurrence of anti-TPO and anti-GPC was found. On the other hand, in people with laboratory signs of thyroid autoimmunity, a higher occurrence of anti-H. pylori antibodies, and also a higher occurrence of anti-GPC autoantibodies was observed. When taking into account gender and age, only differences in anti-GPC in men, especially of higher age, persisted.

The occurrence of anti-GPC autoantibodies in the selected population was 3.8 %. No previous studies were found regarding the anti-GPC prevalence in the general, randomly selected population. All studies describe the occurrence of anti-GPC in various pathological states (Carmel 1992, Annibale et al. 2005, De Block et al. 2003). The occurrence of anti-GPC was not age or gender dependent. Results of this study suggest a possible relationship between anti-GPC and the time course of Helicobacter pylori infection – the occurrence of anti-GPC was higher in H. pylori positive people (6.1 % vs. 1.7 %, p<0.001) and also in people with laboratory signs of autoimmune thyroiditis (7.2 % vs. 3.3 %, p=0.02). H. pylori infection can induce gastric autoimmunity, since of the bacteria leads to the production of antibodies cross-reacting with human gastric mucosa (Appelmelk et al. 1998, Negrini et al. 1991). The gastric mucosal pathogen Helicobacter pylori induces autoantibodies against the gastric proton pump H+,K+-ATPase in 20-30 % of infected patients. The presence of these autoantibodies is associated with severity of gastritis (D’Elios et al. 2004). Our results suggest that gastric autoimmunity caused by H. pylori could also induce thyroid autoimmunity.

To conclude, we would like to point out that this study of a sample of the Czech population showed the occurrence of H. pylori in the same rates as in other developed countries. A slightly lower occurrence of seropositive anti-TPO and anti-Tg autoantibodies was found. Further, the supposed links were confirmed between anti-TPO autoantibodies and anti-GPC autoantibodies, which suggests the involvement of thyrogastroduodenal syndrome type III on one hand and H. pylori seropositivity on the other, as a link between gastric and thyroid autoimmunity.

Conflict of Interest
There is no conflict of interest.

Acknowledgements
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