HELMICOBACTER PYLORI GASTRITIS IN CHILDREN:
A CLINICAL, BACTERIOLOGICAL, AND MORPHOLOGICAL STUDY

MARIKA MIKELSAAR, REET MÄNDAR, HEIDI-INGRID MAAROOS,
SIGNE PÄÄR, TIINA RÄGO, KALLE KISAND, PÄR ALEIJUNG, and
TORKEL WADSTRÖM

SUMMARY

We found a high incidence of Helicobacter pylori infection (83%) among 24 children (2-15 yr.) referred to gastroduodenoscopy because of chronic abdominal pain. Different types of cellular response to H. pylori infection were revealed. The children had mostly (95%) developed mild or moderate chronic infiltration with plasma cells and lymphocytes. In 20% of the cases with somewhat shorter duration of complaints a severe granulocyte infiltration accompanied the chronic inflammation process. Our data suggest the incidence of two types of H. pylori infection in children: chronic and acute. The strains of H. pylori isolated from the children with acute and chronic course of infection exhibited different putative virulence factors, determined by the haemagglutination properties, phospholipase and urease production.

INTRODUCTION

Helicobacter pylori appears as the main aetiological agent of chronic non-specific gastritis (Blaser, 1990) and is believed to be an important factor in the pathogenesis of peptic ulcer disease (Maaroos et al., 1991a; Marshall et al., 1988). However, the natural course of H. pylori infection still remains obscure.

The epidemiology of H. pylori infections varies according to geographical areas and ethnic groups (Dehesa et al., 1991; Dooley et al., 1989; Klein, 1989; Maaroos et al., 1990; Megraud et al., 1989; Siurala, Sipponen, and Kekki, 1988). The early onset of H. pylori infection is thought to be connected with a high incidence of atrophic gastritis which later in life may lead to gastric cancer (Blaser, 1990).

In Estonia a high prevalence rate of gastritis has been proved by random studies in almost 50% of cases in the young (15-18 yr.) age group (Villako et al., 1982) by the rising recovery rate of H. pylori during adult life (Maaroos et al., 1990). It seems that in Estonia the infection often starts in early childhood, whereas some 58% of children with abdominal complaints had H. pylori in the antrum and/or in the corpus (Maaroos et al., 1991b).
Table 1: Age, sex, and duration of complaints and histology of gastric mucosa in NUD children

<table>
<thead>
<tr>
<th>Histology of mucosa</th>
<th>No. of patients</th>
<th>Age range (yr.)</th>
<th>Mean age (yr.)</th>
<th>Sex</th>
<th>Duration of complaints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mucosa</td>
<td>5</td>
<td>2-14</td>
<td>10.0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mild/moderate chronic infiltration</td>
<td>13/2</td>
<td>4-15</td>
<td>11.3</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Severe granulocytic and chronic infiltration</td>
<td>4</td>
<td>4-14</td>
<td>8.8</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

* p<0.05 in comparison with normal mucosa

We have investigated the incidence of *H. pylori* infection among Estonian children (2-15 yr.) suffering from non-ulcer dyspepsia (NUD) and determined some virulence properties of the isolated strains (Wadström et al., 1990).

MATERIALS AND METHODS

The study was carried out at the Children's Clinic of the University of Tartu. Gastric mucosal specimens were obtained from 24 consecutive children undergoing gastrointestinal endoscopy for chronic abdominal pains. The age of the patients (13 male and 11 female patients) ranged from 22 months to 15 year (median 11 yr., mean 10.4 yr.) (Table 1). The 23 patients suffered from NUD and one from celiac disease.

For histological investigation the specimens were taken from the antrum about 1.5 cm above the pylorus and from the middle part of the corpus. The specimens for bacteriological investigation were taken from the antrum, in one case also from the corpus, and put into tubes containing CO₂ and closed with rubber stoppers. The specimens were brought to the laboratory within 2 hours.

Histological studies

Two specimens from each patient were fixed overnight in neutral buffered formalin and subsequently embedded in paraffin; the tissue sections were stained with haematoxylin and eosin and by the Giemsa method for histological evaluation. The gastric antral and corpus specimens of the mucosa were evaluated according to the principles of Sydney classification (Misiewics et al., 1990).

Microbiological investigation

The specimens were washed vigorously on a magnetic rotator in pre-reduced phosphate buffer (PB), homogenised and serially diluted (10⁻²-10⁻⁸) under a stream of CO₂. Aliquots (0.05 ml) of various dilutions of
The properties of five \textit{H. pylori} strains were investigated. Three strains were isolated from 2 children: E6 from the antrum of a 13 year old child with a moderate chronic infiltration of the mucosa; E10 from the antrum and E11 from the corpus of the 6 year old girl with a severe granulocytic and a moderate chronic infiltration. For comparison, we also studied the characteristics of the reference strain (17874) and of one strain E24 isolated from an adult patient with chronic gastric ulcer.

For haemagglutination (HA) bacteria were washed and resuspended in PB (0.15 M, pH 7.2) up to the final concentration of approximately \(10^{10}\) cells/ml. Haemagglutinating properties of the \textit{H. pylori} strains were tested with erythrocytes of the horse, rabbit, guinea pig, bovine, sheep, dove and human. The erythrocytes were washed twice and resuspended in PBS to a 2\% (vol./vol.) suspension and then HA was performed on glass slides by mixing equal volumes (20 \(\mu\)l) of bacteria and erythrocyte suspensions. The reactions were read in 2 minutes time. Two-fold dilutions of bacterial suspensions (25 \(\mu\)l) of strains were made in V-bottomed 96-well plates (Greiner, Sohne, Nyrtingen, FRG) and titrated against erythrocyte suspensions (25 \(\mu\)l) to obtain the minimal bacterial haemagglutinating concentrations. To get even mixing, the plates were agitated, incubated at 20\(^\circ\)C on an orbital shaker for 30 min and allowed to settle before reading. Of the test inhibitor, 30 \(\mu\)l was added to 15 \(\mu\)l of each selected bacterial concentration (dilution before the minimal agglutination concentration). For control wells, 30 \(\mu\)l PBS was used instead of the inhibitor. After incubation at 20\(^\circ\)C on an orbital shaker, 15 \(\mu\)l of the erythrocyte suspension (4\%) was added and the plates were treated in a similar way (Wadström, 1991; Guruge, Ljungh, and Wadström, 1992).

The production of enzymes by \textit{H. pylori} strains for hydrolysis of phospholipides, milk proteins and starch were determined using Egg Yolk, Starch and Skim milk agar plates.

For urease titration bacteria from 48 h culture were suspended in PBS to a final concentration of approximately \(10^{10}\) cells/ml as measured by optical density and centrifuged 2000 x g for 15 min. Two-fold dilutions of supernatant were made in PBS and 0.1 ml of each were inoculated onto urease medium. Reactions were read after overnight storage at 20\(^\circ\)C.

Partition behaviour in an aqueous two-phase system was established with

<table>
<thead>
<tr>
<th>Biopsy data</th>
<th>Total</th>
<th>No. of children \textit{H. pylori} positive:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>bacteriologically</td>
</tr>
<tr>
<td>Normal mucosa</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Chronic infiltration</td>
<td>19</td>
<td>1</td>
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</tbody>
</table>

\begin{table}
\centering
\caption{Prevalence of \textit{H. pylori} infection and histologic gastritis in children}

\begin{tabular}{llll}
\hline
Biopsy data & Total & No. of children \textit{H. pylori} positive: \\
& & bacteriologically & histologically & both \\
\hline
Normal mucosa & 5 & 0 & 0 & 1 \\
Chronic infiltration & 19 & 1 & 2 & 16 \\
\hline
\end{tabular}
\end{table}
Table 3: Haemagglutination titres of H. pylori strains with erythrocytes of various animal species

<table>
<thead>
<tr>
<th>Strains</th>
<th>Rabbit</th>
<th>Horse</th>
<th>Guinea pig</th>
<th>Bovine</th>
<th>Sheep</th>
<th>Dove</th>
<th>Human</th>
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</thead>
<tbody>
<tr>
<td>17874</td>
<td>8</td>
<td>32</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>E 24</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<tr>
<td>E 11</td>
<td>8</td>
<td>32</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>16</td>
<td>2</td>
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<tr>
<td>E 10</td>
<td>1</td>
<td>4</td>
<td>+/-</td>
<td>+/-</td>
<td>4</td>
<td>2</td>
<td>4</td>
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<tr>
<td>E 6</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Titres are expressed as reciprocals of endpoint dilutions.

PEG-palmitate/Dextran as well as with PEG/Dextran-sulphate compounds (Aleljung et al., in press).

Chemicals
Orosomucoid (a-1-glycoprotein concentrate from human plasma) was a kind gift from the Scottish National Blood Transfusion Association, Protein Fraction Centre, Edinburgh. N-acetyl-D-galactosamine, α-L(-)-fucose, N-acetylneuramin-lactose, human transferrin, porcine gastric mucin, asialomucin from bovine submaxillary gland and gangliosides from bovine brain (Type II) were purchased from Sigma Chemical Co., St. Louis, Mo.; Dextran sulphate from Pharmacia; PEG-palmitate was a kind gift from Gote Johansson, Dept. of Biochemistry, University of Lund. Bovine albumin (fraction V), lactoferrin of bovine and human milk were purchased from KEBO LAB, Stockholm.

Statistics
The Fischer's exact test (Runyon, 1977) was used for the comparison of the form of gastritis, the mean age of patients and the duration of complaints.

Ethics
The parents of the children involved were informed of the necessity of gastric biopsy.

RESULTS
The histological and bacteriological findings of the antral mucosa
A subject was considered to be infected with H. pylori only if the bacterium was cultured from or visualised in biopsy specimens. The histology of the antral mucosa was normal in 5 patients. Mild chronic lymphocyte and plasma cell infiltration was found in 13 and moderate in 2 patients (Table 1). Severe infiltration with neutrophils accompanied by mild (1 case) and moderate (3 cases) chronic infiltration was revealed in 4 (20%) children. These children also showed the shortest duration of complaints.
**Table 4: Inhibition of *H. pylori* haemagglutination by glycoconjugates and carbohydrates**

<table>
<thead>
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<td>bo</td>
<td>hu</td>
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</tr>
<tr>
<td>17874</td>
<td>Rabbit</td>
<td>-**</td>
<td>- (+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Horse</td>
<td>-</td>
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<td>(+)</td>
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<tr>
<td></td>
<td>Guinea pig</td>
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<tr>
<td></td>
<td>Bovine</td>
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<tr>
<td></td>
<td>Sheep</td>
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<tr>
<td></td>
<td>Dove</td>
<td>+</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E24</td>
<td>Rabbit</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-(/+)</td>
</tr>
<tr>
<td>E10</td>
<td>Horse</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>(+)</td>
<td>+</td>
<td>-(/+)</td>
</tr>
<tr>
<td>E6</td>
<td>Sheep</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dove</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E11</td>
<td>Dove</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td></td>
<td>Others</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>


(p<0.05) and they were also somewhat younger.

In 20 children (83%) *H. pylori* was detected. In 17 of them the diagnosis of *H. pylori* infection was confirmed both bacteriologically and histologically. In one only the culture and in two only the microscopy of the mucosa proved positive. Only one out of the five cases of NUD who had the normal histological finding of the antral mucosa harboured *H. pylori*. In contrast, all the cases of chronic inflammation of the gastric mucosa had *H. pylori* infection (Table 2).

**The properties of the strains**

The reference strain (17874) and the strain E11 agglutinated in high titres all kinds of erythrocytes used by us. The three other strains did not agglutinate guinea pig and bovine erythrocytes (Table 3). Various carbohydrates and glycoconjugates showed different inhibitory effect of this haemagglutination among the investigated strains (Table 4). HA of strain 17874 was inhibited by orosomucoid, NanLac, and also partially by gangliosides and human lactoferrin. We could not inhibit the HA of strain E11 by any of the substances. HA of the other three strains from an adult patient with peptic ulcer and 2 strains from a child's antrum were inhibited by different inhibitors. We could not find any inhibitory effect of human transferrin, bovine albumin, N-acetyl-D-galactosamine, and α-L(-)-fucose.

All the strains were able to hydrolyse milk proteins (Table 5). In addition, strain E11 and reference strain 17874 agglutinated in high titres all kinds of erythrocytes used by us. The three other strains did not agglutinate guinea pig and bovine erythrocytes (Table 3). Various carbohydrates and glycoconjugates showed different inhibitory effect of this haemagglutination among the investigated strains (Table 4). HA of strain 17874 was inhibited by orosomucoid, NanLac, and also partially by gangliosides and human lactoferrin. We could not inhibit the HA of strain E11 by any of the substances. HA of the other three strains from an adult patient with peptic ulcer and 2 strains from a child's antrum were inhibited by different inhibitors. We could not find any inhibitory effect of human transferrin, bovine albumin, N-acetyl-D-galactosamine, and α-L(-)-fucose.

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Table 5: Enzyme activity of *H. pylori* strains on agar plates

<table>
<thead>
<tr>
<th>Strain</th>
<th>Egg yolk agar</th>
<th>Starch agar</th>
<th>Milk agar</th>
<th>Titre of urease activity**</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 874</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>E 24</td>
<td>-</td>
<td>(+)</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>E 11</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>E 10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>E 6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>1</td>
</tr>
</tbody>
</table>

*+: fermentation; -: no fermentation; (+): weak fermentation.

**Titres of urease activity are expressed as reciprocals of endpoint dilutions.

17874 also expressed fosfolipase activity, and the latter and strain E24 from an adult also had amylase activity.

The reference strain and strain E11 had significantly more urease than the other three strains (Table 5).

By the behaviour of *H. pylori* strains in the two-phase system the strains' cell surface hydrophobicity and charge were investigated. All patients' strains and strain 17874 had low surface hydrophobicity and low negative charge (Figure 1).

Figure 1: Partition behaviour of *H. pylori* in an aqueous two-phase system of PEG-palmitate/Dextran and PEG/Dextran-sulphate (●: Estonian strains; *: strain 17874).
DISCUSSION

This study shows that in Estonian children (2-15 yr.) referred to gastro-duodenoscopy mainly because of chronic abdominal pain, the overall rate of *H. pylori* infection was high (83%). Different cellular response to *H. pylori* infection was revealed. In 95% of cases the children had developed mild or moderate chronic infiltration of gastric mucosa by the Sydney classification system. The infiltrate had no relation to the duration (from 1 month up to 7 yr.) of the complaints. In our study we revealed only one child with *H. pylori* infection whose gastric mucosa was not affected.

Some 20% of the children had developed a severe granulocytic response, accompanied by chronic infiltration. In these patients the duration of complaints was shorter (p<0.05) and the children were somewhat younger.

These data seem to suggest the possibility that there may existing several types of *H. pylori* infection in children: acute and chronic infection. The plasma cell and lymphocyte infiltration is typical for chronic infiltration (Cheng, Irvin, and Costerton, 1989), occurring most commonly among the investigated children. Similar results were obtained by Kilbridge and co-workers (1988) who showed the poor granulocytic response in cases of gastritis in children.

The other type of infection with severe granulocyte infiltration accompanied by chronic infiltration, occurred in cases with a shorter duration of complaints, suggesting the presence of a really acute process in these few children. In literature we could find only few data about the correlation between *H. pylori* caused histological changes in the gastric mucosa and the duration of complaints (Oderda et al., 1990). However, the researchers did not point out the differences in cellular response, depending on the onset of disease.

We suppose that different virulence properties of *H. pylori* strains determined whether the infection that developed was acute or chronic. We therefore investigated comparatively some strains of *H. pylori* isolated from children with acute and chronic gastritis.

In our study the *H. pylori* strains could be divided into two groups. The reference strain and the strain isolated from the gastric corpus mucosa strain (E11) of a child with an acute course of infection expressed the ability both to haemagglutinate more kinds of erythrocytes, produce phospholipase and urease in the highest titres in comparison with the other strains. These properties are probably some of the important virulence factors supporting the cytotoxic effect of *H. pylori* (Cover et al., 1990; Smoot et al., 1990). Unfortunately, we could not follow the cytotoxic production of *H. pylori* strains.

The strains with modestly expressed virulence properties, possessing sialic acid specific haemagglutinins formed the other group. Similar strains of *H. pylori* were described by Wadström (1991). At the same time, our strains' charge and hydrophobicity were quite low, showing that the hydrophobic interactions may not be very important for *H. pylori* to pass through the gastric mucous layer, as was proposed by Rosenberg and co-workers (1986).

Interestingly the two strains isolated from the corpus and antrum of the same child had quite different characteristics. Strain E11, different from the other strain (E10), expressed high urease activity and phospholi-
pase production. Also the putative cell adhesins of strain E11 were different, expressing non-sialic-acid specific haemagglutination. It is interesting to point out that both strains had been isolated from a child whose gastric mucosa expressed both chronic and acute inflammation features. This finding once more suggests the heterogeneity of virulence factor profiles of *H. pylori* strains.

Our earlier results about the varying colonisation rate of the gastric mucosa with *H. pylori* in NUD patients with superficial gastritis may also reflect the different virulence properties of different *H. pylori* strains (Mikelsaar et al., 1990). The idea that there exist *H. pylori* strains with different virulence was recently confirmed by the investigations of Crabtree and co-workers (1991). The authors described the *H. pylori* strains with 120 kDa surface proteins, associated with severe active gastritis and peptic ulceration.

Our data about the high frequency of *H. pylori* infection in children with abdominal pain are mainly in accordance with earlier investigations, showing high frequency of *H. pylori* infection among Estonian children (Maaroos et al., 1991b). The somewhat higher (83% versus 58%) incidence of infection can probably be explained by the large numbers of patients hospitalised for abdominal pain. Our data are also quite close to a Russian study recording the *H. pylori* recovery rate at 55%, and an US study with 65% of infection (Aleksandrova et al., 1989; Westblom et al., 1992).

We conclude that *H. pylori* associated and histologically confirmed gastritis is very frequent (83%) in hospitalised Estonian children (2-15 yr.), endoscopied for abdominal pain. The strains of *H. pylori* isolated from children with two different types (acute and chronic) of cell response to *H. pylori* infection, expressed the heterogeneity of virulence factor profiles. Whether the course of disease of these patients varies later or is similar will be the subject of a further study.

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Marika Mikelsaar, Reet Mündar, and Signe Pälär, Laboratory of Clinical Microbiology, Institute of General and Molecular Pathology, University of Tartu, Tartu, Estonia.

Ralle Kisand, Laboratory of Immunology, Institute of General and Molecular Pathology, University of Tartu, Tartu, Estonia.

Heidi-Ingrid Maaroos, Department of Internal Medicine, University of Tartu, Tartu, Estonia.

Tiina Rägo, Children's Clinic of the University of Tartu, Tartu, Estonia.

Par Aleljung, Torkel Wadström, Department of Medical Microbiology, University of Lund, Lund, Sweden.
LITERATURE


