Peptic ulcer disease in south Ethiopia is strongly associated with Helicobacter pylori

Thor-Henrik Henriksen1,2, Gunnar Nysetør1, Tesfaye Madebo1, Degefe Setegn1, Øystein Brorson4, Tedla Kebede5 and Arnold Berstad5
1 Yirga Alem Hospital, P.O. Box 70, Yirga Alem, Ethiopia; 2 Department of Microbiology, Vestfold Sentralsykehus, 3116 Tønsberg, Norway; 3 Division of Gastroenterology, Medical Department, Haukeland University Hospital, 5021 Bergen, Norway

Abstract
Helicobacter pylori infection was detected in 93% of 174 patients with a peptic ulcer compared with 63% of 116 patients with normal findings ($\chi^2 = 37.3; P < 0.001$) in a cohort of 834 consecutive patients examined by gastroscopy at Yirga Alem Hospital in south Ethiopia. Fourteen patients were given 14 days' treatment with metronidazole 500 mg t.i.d., doxycycline 100 mg b.i.d. and bismuth subnitrate mixture 150 mg q.i.d. Of 10 patients who returned for follow-up, only 2 patients were free from $H.\; pylori$ and cured. Nineteen strains of $H.\; pylori$ from 19 consecutive patients in the same hospital were tested for resistance in vitro against metronidazole, doxycycline and ampicillin. All but 1 were highly resistant to metronidazole; 2 were fully and 14 intermediate resistant against doxycycline. All strains were fully sensitive in vitro to ampicillin. Thus, peptic ulcer was strongly associated with $H.\; pylori$ in south Ethiopia, but eradication of the infection was hampered by antibiotic resistance.

Keywords: Helicobacter pylori, peptic ulcer, incubation time, duodenitis, gastritis, prevalence, epidemiology, treatment, drug resistance, Ethiopia, metronidazole, doxycycline, tetracycline, ampicillin

Introduction
The association between Helicobacter pylori infection and peptic ulcer disease is accepted in the western world. However, $H.\; pylori$ has not been accepted by several authorities as a causative agent of peptic ulcer disease in Africa (HARRIES et al., 1992; HOLCOMBE et al., 1994; RAUWS & TTYTGAT, 1995; TSEGA et al., 1996), although TEDLA (1992) has reported a strong association between duodenal ulcer and $H.\; pylori$ in south Ethiopia. Between 1992 and 1995, 834 patients were referred to gastroscopy in Yirga Alem Hospital (former Sidamo Regional Hospital) in south Ethiopia. The hospital's primary catchment area consists of about 1.5 million inhabitants, but patients who came for endoscopy were recruited from a much larger population. Indications for endoscopic examination were epigastric pain (94%), vomiting (49%), weight loss (22%), bleeding (21%) and dysphagia (14%). Both squamous cell carcinomas and adenocarcinomas were found and most malignancies were located in the lower part of the oesophagus or in the upper part of the stomach, often including the cardia. An analysis of such malignancies has been reported previously (MADEBO et al., 1994).

We now report on the relationship between $H.\; pylori$ infection and peptic ulcer in this cohort, and discuss the magnitude of the problem. We also present the results of a small study on in vitro resistance patterns of $H.\; pylori$ in south Ethiopia, as well as results from the first reported trial of eradication treatment in Ethiopia.

Patients and Methods
$H.\; pylori$ status was determined using Löffler-stained smears and the rapid urease test on biopsy material from 174 of 176 patients with a proven ulcer and 116 of 186 patients with normal findings from among 834 patients examined by gastroscopy at Yirga Alem Hospital in 1992–95. As specificity and sensitivity have been found to be 80–98% and 93–99%, respectively, for the biopsy urease test, and 93–98% and 95–100%, respectively, for histology and Löffler-stained biopsy smears (NYSWTER et al., 1992; MEGRAUD, 1995a; BERSTAD et al., 1996; DUNN et al., 1997) patients were regarded as $H.\; pylori$ positive when any one of these tests was positive. The modified Löffler method for staining of smears that we used was first to squeeze a biopsy specimen well between 2 microscopy slides. After the smears had been allowed to dry, they were fixed for 2 min with methanol, and then stained for 1 min with methylene blue (NYSWTER et al., 1992).

Indications for anti- $H.\; pylori$ treatment were a history of dyspepsia for at least 2 years, and identification of a duodenal ulcer with a diameter of at least 0.5 cm in $H.\; pylori$-positive patients. After having obtained the patient's informed consent, the following 14 days' treatment regimen was offered: metronidazole 500 mg t.i.d., doxycycline 100 mg b.i.d. and bismuth subnitrate mixture 150 mg q.i.d.

Bacterial culture of biopsies from the antrum and corpus of the stomach was performed on a self-made standard charcoal medium (ST) as described elsewhere (HENRIKSEN et al., 1995). The Petri dishes were incubated at 37°C in air-tight plastic jars, in a microaerobic atmosphere produced by Oxoid's gas-generating kit BR38 (Oxoid, England) without the use of a catalyst. Clinical strains from 19 consecutive patients in Yirga Alem Hospital identified as $H.\; pylori$ according to standard criteria (HENRIKSEN et al., 1995) were subcultured and tested for in-vitro resistance against metronidazole, doxycycline and ampicillin. The incubation time was 3 days. For comparison, exactly the same method was used for in-vitro resistance testing of 19 random Norwegian strains of $H.\; pylori$. The same (ST) medium was used throughout the study using the E-test (CEDERBRANT et al., 1993).

However, as warnings have been made concerning the use of charcoal-containing media for sensitivity testing, readings on this medium were compared with readings on other media. For metronidazole, it has been documented that readings on our charcoal medium are in accordance with readings on other media (HENRIKSEN et al., 1996). We performed a similar study to compare in-vitro sensitivity readings for doxycycline on the ST charcoal medium and on an ordinary chocolate medium using 20 Norwegian clinical strains. We used 0.5 Macfarland suspensions, and the incubation time was 3 days. This comparison showed a linear relationship between readings on the 2 media (slope = 0.557, Pearson's (r) correlation coefficient = 0.760). Accordingly, the minimal inhibitory concentration (MIC) for doxycycline of each isolate in this study was calculated by multiplying the E-test readings on the charcoal medium by the factor 0.56.

The other statistical calculations in this paper were all performed by the $\chi^2$ test with Yates' correction.
Results

The gastroscopy findings for the 834 patients examined at Yirga Alem Hospital are shown in Table 1. The prevalence of *H. pylori* infection was significantly higher for patients with a proven ulcer (161 (93%) of 174) than for patients with normal gastroscopy findings (73 (63%) of 116) ($\chi^2 = 37.3; P < 0.001$).

<table>
<thead>
<tr>
<th>Finding</th>
<th>No. of patients (%)</th>
</tr>
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<tbody>
<tr>
<td>Normal$^b$</td>
<td>186 (22)</td>
</tr>
<tr>
<td>Oesophagitis</td>
<td>79 (9)</td>
</tr>
<tr>
<td>Oesophageal carcinoma</td>
<td>73 (9)</td>
</tr>
<tr>
<td>Gastritis/duodenitis</td>
<td>262 (31)</td>
</tr>
<tr>
<td>Gastric or pyloric ulcer$^a$</td>
<td>60 (7)</td>
</tr>
<tr>
<td>Gastric or pyloric cancer</td>
<td>53 (6)</td>
</tr>
<tr>
<td>Duodenal ulcer$^a$</td>
<td>133 (16)</td>
</tr>
<tr>
<td>Pyloric stenosis</td>
<td>111 (13)</td>
</tr>
<tr>
<td>Other, less common</td>
<td>35 (4)</td>
</tr>
</tbody>
</table>

$^a$ *H. pylori* was detected in 73 (63%) of 116 patients with normal endoscopy compared with 161 (93%) of 174 with peptic ulcer.

Fourteen patients were offered and accepted treatment; 10 treated patients returned for a follow-up examination after 2 months. Two of them were cured and *H. pylori* negative. Seven patients were still *H. pylori* positive, although their ulcers were healed or much improved endoscopically. One patient was found to be *H. pylori* negative, but had not improved either clinically or endoscopically, still having his duodenal ulcer.

The results of the in-vitro resistance tests are shown in Table 2. All but 1 (95%) of the Ethiopian strains were resistant to metronidazole compared with only 5 (26%) of the Norwegian strains. All Ethiopian isolates found to be resistant against metronidazole had MICs > 32 mg/L. Only 3 (16%) of the Ethiopian strains were sensitive to doxycycline compared with 11 (58%) of the Norwegian strains. All Norwegian and 18 Ethiopian strains had extremely low MIC values for ampicillin (< 0.016 mg/L). Only 1 Ethiopian strain had a slightly higher MIC value (0.032 mg/L) but was still fully sensitive in vitro.

<table>
<thead>
<tr>
<th>MIC$^a$ (mg/L)</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td></td>
</tr>
<tr>
<td>&lt;8</td>
<td>1</td>
</tr>
<tr>
<td>8–16</td>
<td>0</td>
</tr>
<tr>
<td>&gt;16</td>
<td>18</td>
</tr>
<tr>
<td>Doxycycline</td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>3</td>
</tr>
<tr>
<td>1–4</td>
<td>14</td>
</tr>
<tr>
<td>&gt;4</td>
<td>2</td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
</tr>
<tr>
<td>&lt;0.2</td>
<td>19</td>
</tr>
<tr>
<td>2–64</td>
<td>0</td>
</tr>
<tr>
<td>&gt;64</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ MIC, minimum inhibitory concentration.

**Discussion**

The impression from hospital work in south Ethiopia before the introduction of gastroscopy was that peptic ulcer diseases were uncommon, as found in other parts of Africa (HOLCOMBE et al., 1994; RAUWS & TYTGAT, 1995). Another report from south Ethiopia (TEDLA, 1992) and the present study prove that this is not the case. The observation that 319 (38%) of 834 consecutive patients examined by gastroscopy had cancer or ulcer, 111 (13%) pyloric stenosis and 341 (41%) oesophagitis or gastroduodenitis (Table 1) indicates that these are not rare diseases in south Ethiopia. The apparent change from previous reports could be due to the following. The area is a non-endemic area for the aetiological agent, the recently increased life-span of Africans, or a recent revelation of an existing problem. The observation of TSEGA et al. (1992) that 99% of symptomless carriers of *H. pylori* have full-thickness gastritis is a reminder that we have much to learn about peptic disease in Africa.

In a retrospective study of 444 patients who had been examined by gastroscopy in Arba Minch Hospital, south Ethiopia, a strong association between duodenal ulcer and *H. pylori* was found ($\chi^2 = 15.8; P < 0.001$) (TEDLA, 1992). Adding the information from the present study, we conclude that there is evidence for a strong association between *H. pylori* infection and peptic ulcer disease in south Ethiopia.

The proportion of our ulcer patients who were *H. pylori* positive (93%) is very high, considering the fact that we used diagnostic methods whose sensitivity could be less than 95% (NYSJÖRTER et al., 1992; MEGRAUD, 1995a; BERSTAD et al., 1996; DUNN et al., 1997). The real infection rate in our area is therefore probably very close to 100% for ulcer patients.

Most African populations still have much higher death and birth rates than the peoples of western countries. In modern African populations the mean age is below 20 years, which is about half the estimate for most European countries (WHO, 1995). In addition to this relative lack of aged people in Africa, the relatively low number of peptic ulcers among those infected with *H. pylori* may be due to the continuous high input of newly infected children and adolescents. While being well below 1% per year in northern Europe, the annual infection rate is around 10% in many African countries (RUDELLI et al., 1996; LINDKVIST et al., 1996; PELSER et al., 1997). This is important because the incubation phase for duodenal ulcer is so long that patients are infected during childhood and adolescence, and ulcers are nearly always found in adults (MEGRAUD, 1995b; SULLIVAN, 1995). Duodenal ulcers are also almost exclusively found in patients who have had time to develop chronic antral gastritis (MAGNUS, 1992; SCHROGGER et al., 1967; RAUWS & TYTGAT, 1991). One study has demonstrated that duodenal ulcers can occur at an age which is about 10 years lower in Ethiopia compared with western countries (TSEGA & MENGISTU, 1985). This difference is explained by the observed age difference of almost 20 years for the general populations in the areas compared (WHO, 1995) and the low age at which Ethiopians are infected.

In western countries, about 10% of those who are infected will develop a duodenal ulcer (SIPPONEN et al., 1990; LAM, 1993). Because the demographic and epidemiological conditions are different in Africa, we would expect less than 5% of those infected to have an ulcer during their lifetime in many African countries. Therefore, relatively low rates of duodenal ulcers should be expected in parts of Africa, without any need to doubt the causal relationship between the infection and the disease. The failure of previous studies to identify the relationship between *H. pylori* infection and peptic ulcer disease in Africa can be explained by lack of statistical power. When *H. pylori* infection rates for ulcer patients and controls are 94% and 77%, respectively, the required sample size is 150. When these infection rates are 94% and 87%, respectively, the required sample size is 600 (ALTMAN, 1996). Even where the total sample size is almost adequate, the number of ulcer patients is often far too low (HARRIES et al., 1992; HOLCOMBE et al., 1994; TSEGA et al., 1996).

The poor outcome of our triple drug treatment attempt can be explained by the high rate of metronidazole resistance in vitro, as found elsewhere in developing
countries (HARRIES et al., 1992; BANATVALA et al., 1994), and also a considerable rate of resistance in vitro against doxycycline. In addition, doxycycline has been found to be inferior to tetracycline in the management of H. pylori infections (BOROUDY et al., 1992; HUNT, 1995). We were unable to make a decision to advise tetracycline. However, those patients who returned for follow-up had demonstrated that they had the will and the capacity for cooperation, and we accepted their word that their medication had been taken properly.

The consequences of not challenging the present H. pylori situation in Africa may be dramatic. The African population is in urgent need of further research, not only to identify efficient, cheap and convenient treatment combinations, but also to understand the ways in which H. pylori is being spread.

References


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