

Helicobacter pylori Infection-Current Status in Singapore

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Abstract

Helicobacter pylori infection is a common infection in Singapore affecting about 31% of the population. The seroprevalence of *H. pylori* infection increases with age from 3% in children below 5 years to 71% in adults above 65 years. Amongst the races, Chinese and Indians had similar rates of seropositivity (34.3% and 33.6%) while in Malays it was significantly lower (13.7%; $P < 0.05$).

H. pylori infection is associated with peptic ulcer disease (both duodenal and gastric ulcer) as well as gastric cancer [adenocarcinoma, early gastric cancer and mucosa-associated lymphoma of T cell type (MALT) lymphoma]. Its role in non-ulcer dyspepsia is controversial. *H. pylori* was found in 31% of non-ulcer dyspepsia patients in Singapore compared with 28% in normal healthy controls. Gastric emptying test using indigestible solids shows that gastroparesis per se, *H. pylori* in the presence of gastroparesis (but not *H. pylori* alone) are related to dyspeptic symptom. *H. pylori* plays a synergistic role with non-steroidal anti-inflammatory drugs (NSAIDs) in causing bleeding in gastric ulcer but not in duodenal ulcer. Invasive and non-invasive methods are available for diagnosis of *H. pylori* and should be used to establish the aetiology of gastro-duodenal disease. Currently two groups of therapeutic regimes with eradication rates of 90% are available: bismuth containing regimes and proton-pump inhibitors based regimes. Triple therapy for one week (using three drugs effective against *H. pylori*) is currently the treatment of choice.

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Introduction

Since its discovery in the 1980s by Barry Marshal and Warren Robin, it is realised that *Helicobacter pylori* infection is one of the commonest microbial infections in the world, affecting about 50% of the world population. Infection with *H. pylori* leads to chronic gastritis which leads to the development of gastroduodenal diseases notably peptic ulcer disease and gastric cancer. Infection with *H. pylori* does not however always lead to clinical disease, and only a small proportion of those affected develop peptic ulcer disease or gastric cancer. World-wide, much research has gone into the epidemiology, pathology, diagnosis and pathogenesis of *H. pylori* induced gastritis. This paper highlights some of the work that has been done locally, with a brief review on specific topics, that in the author's view, represent some of the more important recent developments in Helicobacteriology.

Epidemiology

The age prevalence of *H. pylori* infection in different communities appears to fall into two broad groups. In group one, there is a high incidence of acquisition of *H.*

pylori during childhood such that by the age of 20 years, 80% of the population are infected.¹ The population of developing countries show this pattern of *H. pylori* prevalence. In Thailand, for example, in a rural population at a Bangkok orphanage, 74% of children between 1 to 4 years of age were seropositive.²

In group two, the prevalence of *H. pylori* infection in a community is low in childhood but increases with age and 40% of the adult population are infected.³ For example, the prevalence of *H. pylori* infection in the population of California, USA in 1989⁴ was found to be 10% in those between the ages of 18 and 29 years, and rose to 47% in those between the ages of 60 and 69 years. Other developed communities show a similar pattern of prevalence of *H. pylori* infection.

In Singapore, the prevalence of *H. pylori* infection in the population is determined using a serological test (IgG) employing the enzyme-linked immunosorbent assay (ELISA) method. Two thousand, six hundred and twenty-six persons aged between 6 months and 70 years were tested of whom 31.4% (824) were positive. No gender difference was noted. Among the 3 major ethnic groups, Chinese and Indians had similar rates of serop-

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ositivity (34.3% and 33.6%) while the prevalence of *H. pylori* infection in Malays was lower (13.7%; $P < 0.05$). The seroprevalence of *H. pylori* infection increases progressively with age from 3% in children below 5 years of age to 71% in adults above 65 years old. This study shows that the prevalence of *H. pylori* infection in Singapore has a pattern similar to that found in developed countries.⁵ The difference in the age prevalence rates between the Chinese, Indians and Malays was found only in those aged 15 years and above. Among the young (up to 15 years) there was no difference between the 3 different races. This suggests a birth cohort effect rather than a difference in susceptibility to infection. Similar ethnic differences has been observed in Malaysia.⁶

Disease Association

The diseases that are currently associated with *H. pylori* infection are:

- 1) Peptic ulcer disease-duodenal ulcer and gastric ulcer.⁷
- 2) Gastric cancer-adenocarcinoma, early gastric cancer and mucosa-associated lymphoma of T cell type (MALT-lymphoma).⁸

Non-Ulcer Dyspepsia

The role of *H. pylori* in the pathogenesis of non-ulcer dyspepsia is controversial.⁹ There have been several studies comparing the prevalence of *H. pylori* infection in dyspeptic patients and asymptomatic controls. The majority of studies to date show the prevalence of *H. pylori* infection in non-ulcer dyspeptic subjects is higher than in controls.¹⁰⁻¹³ Our own study comparing healthy volunteers with patients with non-ulcer dyspepsia showed that *H. pylori* was present in 28% of healthy controls compared with 31% of non-ulcer dyspeptic patients. The difference was not statistically significant.¹⁴ From the same study, it was found that delayed gastroparesis demonstrated by gastric emptying test using indigestible solids was associated with symptoms of non-ulcer dyspepsia (odds ratio 2.5) whereas *H. pylori* infection did not appear to be related to non-ulcer dyspepsia. However, in the presence of gastroparesis, *H. pylori* infection appeared to be related to non-ulcer dyspepsia (odds ratio 4.1). This study if confirmed, would shed light on the relationship between *H. pylori* infection and non-ulcer dyspepsia.

H. pylori NSAIDs and Bleeding Peptic Ulcer Disease

H. pylori infection is responsible for approximately 95% of duodenal ulcers and 84% of gastric ulcers. Non-steroidal anti-inflammatory drugs (NSAIDs) are responsible for the majority of the remaining cases of peptic ulcer disease. Several investigators reported that the prevalence of *H. pylori* infection in bleeding duodenal ulcer (71%) was lower than in non-bleeding duodenal

ulcer (93%; $P < 0.01$) and no difference was observed in infection prevalence amongst patients with bleeding and non-bleeding gastric ulcer.¹⁵ In contrast, the local experience was different. The risk of bleeding was 63.7% with NSAIDs alone and was 44% with *H. pylori*. However, when both *H. pylori* and NSAIDs were present, the risk of bleeding was 71.8% and the interaction of *H. pylori* and NSAID was statistically significant ($P = 0.025$) suggesting that there is synergism between *H. pylori* and NSAIDs in causing bleeding in patients with gastric ulcer.¹⁶ This study supports the recommended practice of treatment of NSAID-associated ulcers: that *H. pylori* and NSAIDs should be treated independently. Another study of ours which will be presented at DDW (Digestive Disease Week, 1997) showed that *H. pylori* and NSAIDs do not increase the risk of bleeding in patients with duodenal ulcer.

H. pylori Exotoxins and Gastroduodenal Disease

Although both duodenal and gastric ulceration usually occur in the presence of *H. pylori* infection, most infected subjects may never be affected by peptic ulcer disease. The odds ratio of gastric ulceration in infected subject has been found to be 3.2 (95% CI: 1.6 - 6.5) and the odds ratio for duodenal ulceration was 4.0 (95% CI: 1.1 - 14.2).¹⁷ The risk of developing peptic ulcer disease during long-term (>10 years) follow up of *H. pylori* infected subjects varies between 10% and 20% which is approximately a 4- to 10-fold increase over non-infected subjects.¹⁸ The issue of why infection with *H. pylori* leads to peptic ulceration (or gastric cancer) in some people, but not in others, has not yet been solved. Possible explanations that have been put forward include ulcerogenic (carcinogenic) strains, host factors, age of acquisition of infection and interaction with other ulcerogenic (carcinogenic) factors.

Amongst the bacterial factors thought to cause disease, Vac A toxin and Cag A protein are the most studied. In 1988, Leunk et al¹⁹ found that culture supernatants from a subset of *H. pylori* strain could induce vacuole formation in a variety of eukaryotic cells.¹⁹ About 50% of *H. pylori* strains express this activity and other researches find that *H. pylori* infected persons possess neutralising antibodies in their serum to this toxin. The gene encoding the toxin was cloned and named gene Vac A. Vac A gene could be further subdivided according to the middle region (m_1 and m_2) and the signal sequence (s_{1a} , s_{1b} and s_2).²⁰ All s_2 strains were Cag A negative and all Cag A positive strains were s_1 . There were also five Cag A negative strains that were s_1 . s_1 strains of *H. pylori* were found in 91% of people with past or present peptic disease but only in 45% of patients with gastritis alone,²⁰ suggesting that there is a strong association between Vac A genotype and the likelihood of peptic ulceration.

Cag A

In the late 1980s, several groups of investigators noted that a subset of strains (varying between 60% and 80% depending on the study population) expressed a protein migrating between 120 and 130 KDa²¹⁻²³ which have been named Cag A proteins. About 40% to 60% of patients with gastritis are^{21,22} infected with Cag A strains compared with 80% to 100% of patients with duodenal ulceration. For patients with gastric ulceration the results are intermediate. More recent studies have shown an association between infection with Cag A positive strains and gastric carcinoma.^{24,25} *In vitro*, Cag A positive strains induce substantially greater IL-8 production by gastric epithelial cells than the Cag A negative strains, suggesting that Cag A positive strains produce more inflammation than Cag A negative strains. About 60% of *H. pylori* strains found in developed countries are Cag A positive but in developing countries the Cag A positive proportion appears higher. In Singapore, 74% of patients with gastritis were Cag A positive compared to 73% of patients with duodenal ulcer and 73% patients with gastric ulcer.²⁶

Two new genes upstream of Cag A termed pic A and pic B²⁷ have been further identified. There is as yet no work done on pic A and pic B locally.

Diagnosis

It is important to understand the reason for testing for *H. pylori* infection. The presence or absence of *H. pylori* infection in a patient with duodenal ulcer is important because eradication of *H. pylori* results in a significant reduction in the rate of recurrence of ulcer from 80% to 10% at the end of one year. On the other hand, the diagnosis of *H. pylori* infection in an asymptomatic person without peptic ulcer disease is only important when one is collecting epidemiological data to establish an association between *H. pylori* and various disease states. In a patient with acute abdominal pain, the presence or absence of *H. pylori* infection is not very helpful as *H. pylori* infection may be unrelated to the pathology causing pain (e.g. hepatic, colonic and pancreatic disease). Therefore, testing for *H. pylori* should not be a substitute for detailed history taking and careful physical examination.

The tests which are available for the detection of *H. pylori* infection can be subdivided as invasive (or direct) if they detect bacteria in biopsies taken during endoscopy and non-invasive (or indirect) when endoscopy is not required and the tests are based on indirect evidence of the presence of *H. pylori*.

The sensitivity of a test is its ability to detect correctly the patients infected with the bacteria while the specificity is its ability to give a negative test for all those not infected. Therefore a "gold standard" is required. Unfortunately no single technique currently used can fulfill

this role and therefore the results of two or more tests that are known to be reliable are used to compare the results of the technique being evaluated. A true positive result is where two or more tests are positive and a true negative where all of the tests are negative.²⁸

Histology

The Sydney system proposes that two biopsies should be taken from each site within the stomach to reduce sampling error. More recently, at the Asian Pacific Association of Gastroenterology (APAGE) meeting in Yokohama, it was proposed that two biopsies each should be taken from greater curvature, lesser curvature, before and after the angularis. The standard haematoxylin and eosin stain can be used but it is advisable to add another stain such as giemsa.

Culture

When culture is used, it is mandatory to use a transport medium to bring the biopsy to the laboratory. Using Brain Heart Infusion Agar (BHIA) supplemented with 7% fresh whole blood, defibrinated horse blood, a recovery rate of 96% was obtained compared with 78% for commercial trypticase soy agar with 5% sheep blood. For growth of the cultures, it is essential to use at least two different media (selective and non-selective) that have been freshly prepared, to grow the biopsy and to incubate at 37°C in a microaerobic atmosphere, with humidity, for at least 10 days. Several experienced authors have reported an excellent level of sensitivity for culture and consider it to be the best method. The specificity of culture is considered the best of all the techniques because once colonies are established, identification of the bacteria can be carried out.

Rapid Urease Test (Clotest)

This test detects the presence of *H. pylori* quickly. The sensitivity and specificity are linked and depend on the time lapse before the results are read. When read after 1 hour or less the specificity is very good but the sensitivity is not optimal (false negatives can occur); after 24 hours, the sensitivity increases but the specificity decreases because contaminant bacteria harbouring urease can give a false positive result. A false positive result can also be obtained when traces of blood are interpreted as positive. This can occur in 2% to 5% of cases. The sensitivity of the rapid urease test depends upon the number of bacteria present in the biopsy specimen: 10⁴ organisms are required for a positive result. Hence, the test is unsuitable for evaluation of treatment success in post-treatment patients as in patients with failed treatment the number of organisms may not have reached the critical level by 4 weeks. The sensitivity of the test is in the range of 75% to 85% when read at 1 hour and is too low a level of sensitivity for it to be used as the sole method of detection of infection. In the event rapid

urease test is negative, another test should be used. In the local context, clotest has a sensitivity of 77% and specificity of 96%.²⁹

Urea Breath Test

The urea breath test is particularly suited to the detection of *H. pylori* using urea labelled with either ¹⁴C (a radioisotope with a long half-life) or ¹³C (a stable, non-radioactive isotope). The latter test requires a mass spectrometer. The sensitivity and specificity of the test is comparable to that of cultures and histology.³⁰ False positive results can be produced by other urease positive bacteria such as *H. heilmannii* and when the urea breath test is performed just after gastric biopsies. ¹⁴C and ¹³C breath tests have recently been introduced in Singapore.

Serology

Serological tests for diagnosis of *H. pylori* infection rely on detection of IgG antibodies to *H. pylori*. Currently an ELISA is available. A large study³¹ comparing this test with biopsy-based tests showed that a failure of systemic response is noted in only 2% of cases. In children, false negatives can occur up to a few weeks or months before an immune response occurs. Serology is not particularly useful in follow up of patients after eradication because IgG decreases slowly over 3 to 6 months. Use of serology is therefore limited in this clinical situation. Rapid tests (office tests) have a lower sensitivity than the standard ELISA test.

Polymerase Chain Reaction (PCR)

This new technique is extremely sensitive and specific. Most results indicate that the sensitivity of PCR is close to that of culture but for verification of eradication, PCR may be slightly superior.³² This technique is not available locally.

Treatment

Currently the definite indications for treatment of *H. pylori* infection are:

- 1) Peptic ulcer disease caused by *H. pylori*.
- 2) MALT-lymphoma.

Eradication of *H. pylori* in patients with non-ulcer dyspepsia is highly controversial. Most of the therapeutic trials have evaluated symptom status within 1 to 2 months following treatment and the results are equally divided between those that show improvement and those that do not. McCarthy et al³³ followed up 90 patients with non-ulcer dyspepsia for 1 year after treatment with colloidal bismuth citrate, antibiotics (amoxycillin and metronidazole) or a combination of two regimes. Eradication was successful in 49% of patients but improvement in symptoms was comparable at 4 weeks regardless of the change in *H. pylori* status. However, one year later, the dyspeptic scores of patients

who had become *H. pylori* negative and remained negative decreased while the dyspepsia scores rose again to pre-treatment levels in patients whose infection had not been cured. This study, has not, by any means proven conclusively that *H. pylori* eradication is beneficial to patients with non-ulcer dyspepsia. The major weakness of this study is that the patients were aware of the outcome of the eradication treatment at 4 weeks, and therefore could have affected their symptom scores within the next 11 months. A multicentre trial without this weakness is in progress and may shed light on this issue. Nevertheless, the importance of McCarthy's work lies in the fact that following treatment patients may show improvement in dyspeptic symptoms regardless of *H. pylori* status. The working party on *H. pylori* in Singapore is of the opinion, based on current evidence, that it is not justifiable for routine eradication of *H. pylori* in dyspeptic patients.³⁴

Treatment Regimes

Three groups of therapeutic regimes are recommended because they appear to achieve 90% cure rates. They are:

- 1) Bismuth containing regimes.
Bismuth-metronidazole-tetracycline combination.
- 2) Proton pump inhibitors (PPIs) containing regimes—
PPIs plus clarithromycin and either metronidazole or amoxycillin.
- 3) PPIs plus bismuth containing regimes.

A combination of ranitidine plus bismuth subcitrate (together with clarithromycin) has just been approved by the Ministry of Health, Singapore.

Bismuth plus metronidazole and tetracycline (BMT) for one week³⁵ is comparable to 2 weeks treatment and can achieve 85% to 90% cure provided the organism is sensitive to metronidazole and there is good patient compliance. Bismuth-metronidazole-amoxycillin (BMA) combination is less effective than BMT. PPI in combination with clarithromycin or amoxycillin for two weeks (dual therapy) achieved eradication rates of 71% and 54% respectively³⁶ and is not strongly recommended. On the other hand, PPI³⁷ plus two of the following: metronidazole or tinidazole, clarithromycin, amoxycillin for 1 week achieves 85% to 90% cure rates. The combination of PPIs, metronidazole and amoxycillin appears to be the least effective and may require a 14-day treatment period.

Three problems limit the effectiveness of regimes designed to cure *H. pylori* infection. First, antibiotic resistance is encountered with metronidazole and clarithromycin. With metronidazole resistance strains, eradication rates can fall by 20% to 30%. In a study that spanned 2½ years the prevalence of metronidazole-resistant strains increased from 45% in the first year to 70% in the second year and 81% in the last six months.³⁷ Metronidazole resistance of this magnitude may make it necessary to consider using non-metronidazole contain-

ing regimes. Patient compliance is often poor because of complex regimes. With the twice daily dosing used in the latest regimes this problem is solved. Serious adverse effects occur only rarely (0.1% to 0.5%). Reinfection or recrudescence (recurrence of infection after apparent eradication) is not a serious problem as was originally thought and is of the order of 6% locally.³⁸

Conclusion

The story of *H. pylori* is unique in that since its discovery the work that has been done has been carried out by gastroenterologists and clinicians defining its role in the pathogenesis of gastroduodenal disease, formulating therapeutic regimes, while microbiologists hesitated. The next decade should see collaboration of microbiologists and molecular biologists in an attempt to eradicate *H. pylori* infection in man.

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