Prevalence of *Helicobacter pylori* in Sri Lanka as Determined by PCR

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Fifty-seven Sinhalese patients were investigated for the presence of *Helicobacter pylori* by PCR. A prevalence of 70.1%, with 47.5% positive for cagA, was demonstrated. The most common vacA allele was s1am1. There was no significant association between either the s1 allele or the cagA allele and severe gastroduodenal disease. There was an association between the s1 allele and the cagA locus.

*Helicobacter pylori* is one of the most common causes of chronic bacterial infections in humans and is important in the pathogenesis of gastrointestinal disease, such as duodenal ulcer, gastric ulcer, gastric cancer, and lymphoma (1, 2).

Only three studies have reported on the prevalence of *H. pylori* in Sri Lanka, using either culture or a rapid urease test (RUT). In one study using the RUT, a prevalence of 46% was recorded in an adult symptomatic population (5), while in a study using culture and the RUT, only 12% of a symptomatic population aged 18 to 80 years were recorded as colonized (3). More recently, only 2.9% of 67 patients with functional dyspepsia were positive by the RUT (4). In India, a prevalence of 80% has been reported.

Because the *H. pylori* strains prevalent in Sri Lanka have not previously been reported upon, the objectives of this study were to determine their prevalence by using PCR, as it is sensitive (7), gives an indication of current infection, and yields additional information regarding the type of strain colonizing the individual.

Fifty-seven dyspeptic Sinhalese patients were enrolled; 41 were males and 16 were females, with an age range of 4 to 74 years (mean, 44 years). Two gastric biopsy samples were taken from the antrum for histology and DNA studies. The DNA was extracted by using a PuregeneD-5500A kit (Flowgen, Ashby de la Zouch, United Kingdom). PCRs for the 16S rRNA, *glmM* tubes (AB Gene, Epsom, Surrey, United Kingdom). The 16S rRNA gene was detected by using the primers 16S/1 and 16S/2 (5) for 95°C for 2 min (1 cycle) followed by 40 cycles of 95°C for 1 min, 55°C for 1 min, and 74°C for 1 min with a final extension of 74°C for 5 min (10).

Variation within the s1 allele was determined by using the primers SS1-F (5′GTGACGATCACCCAGCAGC3′), SS3-F (5′AGCGCCATACCGCAAGAG3′), SS2-F (5′GCTAACACGCCAAATGATCC3′), and VA1-R (5′CTGTTGAATGCCCAAAAC3′), which generate a 190-bp amplicon for the s1a allele (SS1F and VA1R), a 187-bp amplicon for the s1b allele (SS3F and VA1R), and a 199-bp amplicon for the s2 allele. The cycle parameters were 95°C for 5 min (1 cycle) followed by 35 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min. The cycle parameters were 95°C for 5 min (1 cycle) followed by 35 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min with a final extension of 74°C for 5 min (10).

There was an association between the s1 allele and severe gastroduodenal disease. There was also a significant association between the s1 allele and the *cagA* gene. The prevalence of *Helicobacter pylori* in Sri Lanka has been reported as 80%, with a prevalence of 70.1% in this study.

The presence of the *cagA* gene was demonstrated by using the primers cagA-F (5′TTGACCAACACCAACAAAGAAG3′) and cagA-R (5′CTCCCTTAATGTGGAGATTC3′) (11) for 95°C for 2 min (1 cycle) followed by 40 cycles of 95°C for 1 min, 50°C for 1 min, and 74°C for 1 min with a final extension of 74°C for 5 min, which generates an amplicon of 183 bp. The *Helicobacter* 16S rRNA gene was detected by using the primers JW21-F (5′GGCCTATGTTGTGAGATTC3′) and JW22-R (5′GGTTAGGTGCAATGAGA3′) (6) with 35 cycles of 93°C for 1 min, 55°C for 1 min, and 72°C for 1 min followed by 72°C for 10 min, which amplifies a 139-bp amplicon. The *glmM* gene was detected by using the primers F5′AAGCCTTATGGGGTGTTAGGGTTTT3′ and R5′AAGCTTACCTTAACACCCAGC3′ with 35 cycles of 93°C for 1 min, 55°C for 1 min, and 72°C for 1 min (7), which amplifies a 249-bp amplicon.

Forty-six of 57 (80.7%) of these patients had only gastritis, and *Helicobacter*-like organisms were found in 3 of them; 5 of the 57 (8.7%) had both gastritis and a duodenal ulcer, 2 (3.5%) had gastritis and a gastric ulcer, 1 (1.7%) had gastritis and a gastric carcinoma, and only 3 (5.3%) had normal histology. A total of 40 (70.1%) biopsies were positive for *H. pylori* by 16S rRNA, *glmM*, and vacA gene amplification. Of these 40 positive specimens, 19 (47.5%) were also positive for *cagA*, and colonization by *cagA*-positive isolates did not relate to the

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presence of severe gastroduodenal disease ($P < 0.05$). There was also no correlation between colonization by organisms carrying the s1 allele and severe gastroduodenal disease ($P < 0.05$). The most common allele type was s1am1, followed by s1bm1. Although they were positive by other primers, nine samples could not be fully subtyped using these primers. In six of these nine, the “m” region could not be typed but the “s” region showed 4s1a and 2s1b, both associated with gastritis, and in three, the “s” region could not be typed further than s1 but all were m1. Two biopsy samples demonstrated multiple infection, with more than one subtype of the “m” region present. Of 35 isolates that were vacA s1 (including those that could not be fully subtyped in the signal sequence), 19 (44%) were cagA positive. Of those that could not be fully subtyped, three of four that were s1, one of two that were s1b, and two of three that could be typed only as s1 were cagA positive. The relationship between the possession of the s1 allele and the cagA locus was statistically significant ($P = 0.05$).

We have shown that the prevalence of H. pylori in a dyspeptic Sinhalese population is 75.4% by using PCR. The low prevalence in previous studies may reflect low sensitivity of the methods. The predominant vacA allele is the s1am1 type similar to that found in Northern Europe, but both the s1b and s1c allele types were also represented. This may reflect the multi-ethnic population and historical invasions of Sri Lanka by the English, Portuguese, and Dutch. These data indicate that 45% of the strains were cagA positive, and as in the Far East (9), there was no correlation between either vacA or cagA and severe gastroduodenal disease. There was, however, an association between cagA and the s1 vacA allele, as has been shown in other studies.

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