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Prevalence of *Helicobacter pylori vacA* and *cagA* Genotypes in Ethiopian Dyspeptic Patients

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A total of 300 gastric biopsy samples and 50 *Helicobacter pylori* isolates were collected from Ethiopian adult dyspeptic patients. The *vacA* and *cagA* genes were detected in 90 and 79% of biopsy specimens, respectively, and in 100 and 87% of clinical isolates, respectively. Both genes were detected in 84% of the gastric biopsy samples and in 87% of the clinical isolates. Among *vacA* genotypes, the s1/m1 genotype was the most common in gastric biopsy samples (48%). The *vacA* and *cagA* positive *H. pylori* strains were detected to a higher degree in patients with chronic active gastritis (71%) than patients with other histopathological findings (29%) ($P < 0.05$).

Several *Helicobacter pylori* virulence genes related to the risk of gastroduodenal diseases have been proposed. The vacuolating cytotoxin (*vacA*) gene is present in virtually all *H. pylori* strains and contains at least two variable regions, the signal (s) region, which encodes the signal peptide, and the middle (m) region (4). The s region has been divided into two subtypes, s1 and s2, and the m region has been divided into two subtypes, m1 and m2 (19). The amount of cytotoxin produced is highest with the s1/m1 allele, followed by the s1/m2 allele, while no cytotoxin activity is found when s2/m2 is present (19). The cytotoxin-associated gene (*cagA*) is a marker for a genomic pathogenicity island of 40 kb (6). A significant association between the presence of ulcers or gastric carcinoma and the presence of *vacA* type s1 and *cagA* gene (5, 19). The present study represents the first in Ethiopia to detect *H. pylori vacA* and *cagA* genotypes from gastric biopsy samples and clinical isolates using PCR-based methods.

MATERIALS AND METHODS

Study subjects. A total of 300 consecutive informed and consenting adult patients with dyspeptic symptoms from the gastrointestinal referral and follow-up clinics of Department of Internal Medicine, Tikur Anbassa University Hospital, Addis Ababa, Ethiopia, were investigated for *H. pylori* between November 2000 and August 2002. The mean age of the patients was 36.5 years (standard deviation, 13.8 years; range, 15 to 90 years). The majority of patients (76%) were between the ages of 15 and 44 years. Of the 300 patients, 186 (62%) were males and 114 (38%) were females (resulting in an overall male to female ratio of 1.6:1).

The study was approved by the Department Graduate Committee, the Faculty Research Publications Committee and endorsed by the Faculty Academic Commission and has been ethically cleared.

Culture and identification. Antral gastric biopsy samples were taken from each dyspeptic patient. The biopsy specimens were put into sterile phosphate-buffered saline containing 15% glycerol and immediately transported to laboratory for culture. Biopsy samples for molecular analysis were kept frozen in 15% tryptone soy broth (Oxoid Ltd., Basingstoke, England) and stored at −70°C until analyzed.

H. pylori was cultured from antral biopsy specimens using a standard method (17). *H. pylori* identification was based on morphology, Gram staining, oxidase, catalase, and urease tests. All the isolated *H. pylori* strains were kept frozen at −70°C in the tryptone soy broth medium containing 15% (vol/vol) glycerol until genotyping was performed. The *H. pylori* reference strain (CCUG 17874) (Culture Collection, University of Gothenburg, Gothenburg, Sweden) was cultured throughout the study for quality control.

Histopathology. Gastric biopsy specimens were fixed in 10% formalin and embedded in paraffin. The sections (4 to 5 μm thick) were cut and stained with hematoxylin and eosin (2). The histological findings from the sections stained with hematoxylin and eosin were scored according to the updated Sydney system of classification and grading of gastritis (7).

Genomic DNA extraction. Biopsy specimens and isolates were centrifuged at 10,000 × *g* for 5 min. The DNA was extracted from the pellets by use of the QIAamp DNA kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations and DNA stored at −20°C until analysis. DNA extraction negative controls were performed in parallel by including sterile tubes without samples to check for contamination of the DNA extraction reagents.

PCR-DGGE. The PCR amplification was carried out using a GeneAmp 2700 Thermal cycler (Applied Biosystems, Foster City, Calif.). A seminested *Helicobacter* genus-specific PCR assay targeting the 16S rDNA was used to amplify *Helicobacter* DNA (9). Denaturing gradient gel electrophoresis (DGGE) analysis of the PCR products was performed in a DCode system (Bio-Rad, Hercules, Calif.) as recently described (1). Migration ladder containing PCR products of reference *Helicobacter* strains (*H. muridarum* [CCUG 29262], *H. bilis* [CCUG 38995], *H. pullorum* [NCTC 12825], *H. pylori* [CCUG 17874], "*Flexispira rappini*" [CCUG 23435], *H. hepaticus* [CCUG 33637], and *H. bizzozeronii* [AF 53]) was run in parallel as a mobility ladder.

***vacA* and *cagA* genotyping.** Detection of *H. pylori vacA* and *cagA* genes was performed on gastric biopsy specimens and isolates positive for *H. pylori* by PCR-DGGE as previously described (15, 19). As a positive control, *H. pylori* (CCUG 17874) DNA (~0.1 ng) was added to the reaction mixture, while 5 μl of sterile deionized Millipore-filtered water was added to the reaction mixture as a negative control. Estimation of size of the PCR products was done by using Gene ruler 100-bp DNA ladder (Fermentas, Vilnius, Lithuania). The products of each PCR assay were visualized by electrophoresis in a 1.5% agarose gel containing ethidium bromide (15).

Statistical analysis. Epi info version 2000 (Centers for Disease Control and Prevention, Atlanta, Ga.) was used for statistical analysis. Chi-square or Fisher's exact test was applied to test whether differences between values were significant. *P* values <0.05 were considered statistically significant.

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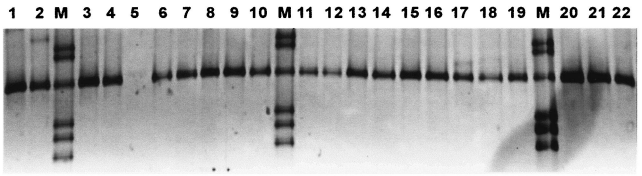

FIG. 1. DGGE analysis of PCR products amplified by using *Helicobacter* genus-specific primers. Lanes, 1 to 4 and 6 to 22, *H. pylori*-positive samples; 5, *H. pylori*-negative sample; M, mobility marker (PCR products of reference *Helicobacter* spp. [from the top: *H. bilis*, *H. pullorum*, *H. pylori*, "*F. rappini*," *H. hepaticus*, and *H. bizozzeroni*]).

RESULTS AND DISCUSSION

Histopathological examinations were performed on 276 (92%) of the gastric biopsy specimens, whereas the remaining 24 (8%) were not adequate (too small). Abnormal findings were observed in all examined specimens. Chronic gastritis was found in 48 (17.4%) patients, chronic active gastritis in 185 (67%), chronic atrophic gastritis in 24 (9%); chronic atrophic gastritis with intestinal metaplasia in 17 (6%) and malignant lesions in 2 (0.6%) patients. The overall prevalence of chronic gastritis was 99.3%. The most common histopathological findings in the present study were similar to those reported from other parts of Africa (10).

The seminested *Helicobacter* genus-specific PCR assay detected *Helicobacter* DNA in 273 of 300 (91%) of the biopsy specimens. DGGE analysis showed that all PCR products have mobility pattern similar to the *H. pylori* reference strain (Fig. 1).

The *vacA* gene was detected in 246 of 273 (90%) of *H. pylori*-positive gastric biopsy specimens, which is similar to reported results from The Netherlands (93%) and Hong Kong (95.8%) (14, 21), emphasizing high sensitivity of the PCR method employed in the present study. The *vacA* genotypes s1/m1, s1/m2, s2/m2, and s2/m1 were found in 48, 28, 9, and 2% of the specimens, respectively (Table 1), whereas, 15 biopsy specimens (6%) were incomplete and thus did not yield a detectable PCR product for the *vacA* s or m regions. The pattern of *vacA* alleles in this study is in agreement with those reported in other studies (3, 8, 16, 18, 20). However, the frequency of *vacA* s1/m1 allelic type in this study is higher than figures reported from The Netherlands (36%), Hong Kong (26 to 31%), and Nigeria (24%), but lower than figures reported from Brazil (80%) and Korea (78%) (3, 11, 16, 20, 21). In the present investigation, the rare *vacA* s2/m1 allele was detected in 4 (2%) of the 246 gastric biopsy specimens examined, also reported in studies in South Africa and Chile (12, 13). Multiple *vacA* genotypes were found in 18 (7%) of the 246-biopsy specimens examined. The most frequent multiple *vacA* genotypes were s1/m1m2 (11 of 18; 61%).

TABLE 1. *H. pylori vacA* genotypes in gastric biopsy samples and clinical isolates

vacA genotype	No. (%) of samples or isolates	
	Gastric biopsy	Clinical
s1/m1	118 (48)	31 (60)
s1/m2	68 (28)	14 (27)
s2/m2	23 (9)	4 (7)
s2/m1	4 (2)	0
s1/m1m2[a]	11 (4)	2 (4)
s2/m1m2[a]	2 (0.7)	0
s1s2/m2[a]	4 (2)	1 (2)
s1s2/m1m2[a]	1 (0.3)	0
Incomplete (*vacA* s or m)[b]	15 (6)	0
Total *vacA* positive	246 (100)	52 (100)

[a] Multiple *vacA* genotypes.
[b] For *vacA* s and m regions, $n = 7$ and $n = 8$, respectively.

TABLE 2. Distribution of *H. pylori vacA* and *cagA* genotypes in 273 gastric biopsy samples and 52 clinical isolates

Genotype status	No. (%) of samples or isolates	
	Gastric biopsy	Clinical
vacA$^+$ *cagA*$^+$	207 (76)	45 (87)
vacA$^+$ *cagA*	39 (14)	7 (13)
vacA cagA	17 (6)	0
vacA cagA$^+$	10 (4)	0

The *vacA* was detected in all 52 *H. pylori* isolates tested (Table 1). The prevalence of the *vacA* subtypes s1/m1, s1/m2, and s2/m2 was 60, 27, and 7%, respectively. Three (6%) of the isolates contained mixed *vacA* subtypes; two s1/m1m2 and one s1s2/m2 from a single *H. pylori* isolate. The multiple *vacA* genotypes detected in this study are similar to results from Italy reported by Blaser and Berg (5). Surprisingly, the prevalence of multiple *vacA* genotypes in this study was much lower compared with results reported from Brazil (13%), Chile (32%), Korea (18%), and The Netherlands (11%) (3, 11, 13, 20). The low prevalence of multiple *vacA* genotypes in a country with a high prevalence of *H. pylori* infections in the general population may be the result of a low number of mosaics of any combination of signal (s) and mid-region (m) alleles of the bacteria circulating in the community.

Of the 273 *H. pylori* PCR-positive biopsy specimens, 217 (79%) were *cagA* positive. Four different genotypic combinations were recognized based on analysis of the positive and negative *vacA* and *cagA* results—*vacA*$^+$ *cagA*$^+$, *vacA*$^+$ *cagA*, *vacA cagA*, and *vacA cagA*$^+$, which were found in 76, 14, 6, and 4% of specimens, respectively (Table 2). Forty-five of the 52 (87%) *H. pylori* strains were positive for both *vacA* and *cagA*, whereas the remaining isolates 7 (13%) were only *vacA* positive (Table 2). Statistical analysis showed no difference in the detection of the *vacA* and *cagA* in gastric biopsy specimens and clinical isolates ($P = 0.70$ for *vacA*, $P = 0.96$ for *cagA*). In addition, no statistical differences in the frequency of detection of the different *vacA* allelic types from gastric biopsy specimens and clinical isolates were found ($P > 0.05$). The prevalence of *cagA* positive *H. pylori* strains varies from one geographic region to another, e.g., 38% in Chile, 48% in Sri Lanka, 67% in The Netherlands, 81% in the United Sates, 90% in Hong Kong, 97% in Korea, 93% in Nigeria and 94% in Brazil (3, 8, 11, 13, 16, 18, 20, 21). Correlation of histopathology results with *vacA* and *cagA* genotypes showed that *vacA* and *cagA* positive strains were detected to a higher degree in patients with chronic active gastritis (71%) compared with patients with other histopathological findings (29%) ($P < 0.05$) (Table 3).

Molecular analyses demonstrated that more than 80% of the

TABLE 3. Distribution of *vacA* and *cagA* allelic types according to histopathological findings in the antra of 276 dyspeptic patients

Histopathological findings[b]	No. (%) with *vacA* and *cagA* results													
	s1/m1		*s1/m2*		*s2/m1*		*s2/m2*		Mixed		Inc[a]		Negative	
	cagA$^+$	*cagA*	*cagA*$^+$	*cagA*	*cagA*$^+$	*cagA*	*cagA*$^+$	*cagA*	*cagA*$^+$	*cagA*	*cagA*$^+$	*cagA*	*cagA*$^+$	*cagA*
Nonatrophic gastritis														
CG (n = 48)	9 (19)	2 (4)	4 (8)	6 (13)	0	0	1 (2)	0	4 (8)	0	3 (6)	0	1 (2)	18 (38)
CAG (n = 185)	80 (43)	2 (1)	36 (20)	4 (2)	2 (1)	0	10 (6)	10 (6)	6 (3)	4 (2)	6 (3)	4 (3)	2 (1)	19 (10)
Atrophic gastrits														
CAAG (n = 24)	9 (38)	1 (4)	10 (42)	2 (8)	0	0	0	0	1 (4)	0	0	0	0	1 (4)
CAAGI (n = 17)	9 (53)	0	3 (18)	0	0	0	0	0	1 (6)	0	1 (6)	0	0	3 (18)
Malignant lesions														
Adenocarcinoma (n = 1)	0	0	0	0	0	0	0	0	1 (100)	0	0	0	0	0
MALT lymphoma (n = 1)	1 (100)	0	0	0	0	0	0	0	0	0	0	0	0	0
Total (n = 276)	108 (39)	5 (2)	53 (19)	12 (4)	2 (1)	0	11 (4)	10 (4)	13 (5)	4 (1)	10 (4)	4 (1.4)	3 (1)	41 (15)

[a] *vacA* gene was incomplete (Inc) to yield a detectable PCR product for the *vacA s* or *m* region.
[b] Abbreviations: CG, chronic gastritis; CAG, chronic active gastritis; CAAG, chronic active atrophic gastritis; CAAGI, chronic active atrophic gastritis with intestinal metaplasia; MALT, mucosa-associated lymphatic tissue.

Ethiopian *H. pylori* strains (detected from dyspeptic patients) harbor both *vacA* and *cagA* genes. The presence of such combined genotypes in infected patients has been proposed to increase the risk for development of clinical complications such as peptic ulcerations and gastric cancer (4, 20). Further genetic analysis should be conducted to determine the homology of the *H. pylori* genome in members of the same family in order to study the transmission of *H. pylori* from person to person.

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