Helicobacter pylori genotypes in Lithuanian patients with chronic gastritis and duodenal ulcer

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Key words: Helicobacter pylori; duodenal ulcer; chronic gastritis; virulence factors; cagPAI; vacA; iceA.

Summary. Objective. Clinical outcome of Helicobacter pylori (H. pylori) infection might be associated with specific virulence-associated bacterial genotypes. The distribution of different bacterial genotypes varies geographically. The aim of this study was to assess the relationship between cagPAI, vacA, and iceA status and severity of the disease in patients from Lithuania, infected by H. pylori.

Material and methods. H. pylori from 81 patients (37 with duodenal ulcer and 44 with chronic gastritis) was isolated from gastric biopsy specimens and cultured. Bacterial genotypes cagPAI, vacA (s and m subtypes) and iceA were analyzed by polymerase chain reaction using specific primers.

Results. The cagPAI was identified in 59.3% of Lithuanian H. pylori strains investigated. H. pylori strains cultured from duodenal ulcer (DU) patients more frequently (P<0.01) contained cagPAI and vacA s1 genotypes (75.7% and 75.7%, respectively) in comparison to isolates from chronic gastritis (CG) patients (45.5% and 40.9%, respectively). Evaluation of nucleotide sequence of the vacA middle-region revealed that vacA s2/m2 genotype was more frequent in CG than in DU patients (56.8% and 24.3%, respectively; P<0.05). We have not found any differences in the frequency of iceA1 genotype between the DU and CG patients (46.0% and 40.9%, respectively; P>0.05).

Conclusion. Our study suggests that cagPAI and vacA s1 genotypes are associated with peptic ulceration in Lithuanian patients infected by H. pylori.

Introduction

Helicobacter pylori (H. pylori) infection is one of the most prevalent human bacterial infections and is associated with different gastroduodenal diseases, such as gastritis, peptic ulcer, and is an important risk factor for the development of gastric cancer and gastric lymphoma (1–4). Several potential markers of pathogenicity have been described in H. pylori, and some of them seem to be associated with more severe clinical outcomes of the infection (5–7). The genetic variability of H. pylori is high, (5, 8–10), and several genes have been identified that may play a role in the pathogenicity (cagA, vacA, iceA). Besides being associated with specific diseases, certain genotypes are more frequently found in certain ethnicities or geographic regions of the world (11–13). In Western populations, H. pylori containing cytotoxin-associated gene (cagA), which is a marker for a genomic pathogenicity island (cagPAI), is more strongly associated with more severe disease than strains lacking cagA (14). In contrast, nearly all East Asian strains carry the cagPAI independent of disease status (15, 16). Similar studies are still scanty in East European region, where prevalence of H. pylori infection is high, and both peptic ulcer disease and gastric adenocarcinoma are very common (17–21). In Lithuania up till now, the genetic characteristics of H. pylori have not been studied yet, except the pilot study by Čalkauskas et al. (22). The present study aimed to analyze cagPAI, vacA, and iceA status directly in gastric biopsy specimens from 81 patients from Lithuania in relation to clinical data.

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Materials and methods

H. pylori strains were obtained from 81 patients (39 males and 42 females; mean age, 50.04±16.35 years; range, 16–88 years) who were referred for upper gastrointestinal endoscopy at Kaunas University of Medicine Hospital due to dyspeptic symptoms. Thirty-seven patients had duodenal ulcer (DU), and forty-one – chronic gastritis (CG). Patients using aspirin or nonsteroidal anti-inflammatory drugs were excluded from the study, and none of the (CG) patients had the history of peptic ulcer disease.

Helicobacter pylori culture

H. pylori was isolated from gastric biopsy specimens and cultured on the surfaces of brain-heart infusion (BHI) agar plates supplemented with 10% horse blood, 0.4% IsoVitaleX, amphotericin B (8 μg/mL), trimethoprim (5 μg/mL), vancomycin (6 μg/mL) and Wilkins Chalgren Anaerobic Agar with 10% horse blood and Dent supplement (Oxoid). The media were incubated under microaerophilic conditions generated by CampyPak-Plus (Becton Dickinson) at 37°C from 3 to 7 days. The identity of the colonies was confirmed by typical Gram's staining and biochemical testing for urease, catalase, and oxidase. Bacterial stocks were maintained at −70°C in Brucella broth (Difco), supplemented with 15–20% glycerol.

DNA assay

Chromosomal DNA was isolated from confluent plate cultures using the QIAamp tissue kit (Qiagen, Chatsworth, Calif.). Specific PCR was generally carried out in 20-μL volumes containing 10 ng of DNA, 1 U of Tag polymerase (Promega, Madison, Wis.), 10 pmol of each primer per reaction, 2 to 3 mM MgCl₂, and 0.25 mM of each deoxynucleoside triphosphate in a standard buffer. Cycling conditions were usually 30 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for a time dependent on the expected product size (1 min per kb). The PCR primers used in this study are listed in Table 1.

Statistical analysis

Chi-square test or Fisher exact test was used for

| Table 1. Oligonucleotide primers used for polymerase chain reaction |
|---------------------------------------------------------------|
| **Genes** | **Primers** | **Sequence** |
| cagPAI right junction | CagF4584 CagR5280 | 5’-GTAAATACAAAGGTGTGTTTCCAAAATC² |
| cagPAI type I and IV | CagF4856 CagR5280 | 5’-GCATGGAAGAAATATCTTACG² |
| cagPAI type II | IS606-1692 CagR5280 | 5’-CTAACAATTTGCCATTATGCTGT² |
| cagPAI type III | Fcn unk CagR5280 | 5’-TGAATATCCTTAATGATCG² |
| cagPAI “empty” site | Luni3 CagR5280 | 5’-ATAGCGTTTTTGTCATAGAATGC² |
| vacAs1 (259 bp) or s2 (286 bp) | VA1-F VA1-R | 5’-ATGAAATACAACAAACACAC |
| vacAs1a | SS1-F VA1-R | 5’-GTCGTTAATGCGCAAC |
| vacAs1b | SS3-F VA1-R | 5’-AGGCACACCCCAAGAG |
| vacAs1m | VA3-F VA3-R | 5’-GGTCAAAAATGCGTCTGG |
| vacAs1m2 | VA4-F VA4-R | 5’-GGAGCCCCAGGAAACATTG |
| iceA1 | IceAF5 IceAR4 | 5’-GTGTATTATACAAATATC |
| iceA2 | IceAF6 IceAR5 | 5’-TTCACCTATTATCCATAGGT |

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statistical analysis of the results, and significance was accepted at P<0.05.

Results

The cagPAI was identified in 59.3% (48/81) of Lithuanian H. pylori strains investigated. Thirty-three cultures, from which no cagPAI-specific PCR product was obtained, yielded an empty-site product of the expected 550-bp size, indicating that they truly lacked the cagPAI. The vacA gene was identified in all of the 81 H. pylori strains. Forty-six of the 81 (56.8%) cultures yielded a 259-bp fragment, indicating vacA s1 alleles, and 35 had a 286-bp fragment, representing vacA s2 alleles. None of the strains yielded a PCR product of any other size. Analysis of the 46 strains with the s1 genotype revealed that 44 (95.7%) of them belonged to vacA s1a and only 2 (4%) to vacA s1b subtypes. The presence of cagPAI showed a strong association with the presence of the vacA s1 allele (Table 2). Investigation of the alleles in the middle region of vacA identified 11 (13.6%) of the 81 H. pylori isolates as m1 type and 54 (66.7%) as m2 type. Alleles in the middle region of 16 strains were nontypable (m? type). The vacA s1/m1 combination was found in 11 (23.9%); s1/m2 in 20 (43.5%); and s2/m2 was present in 34 (97.1%) of typable strains. The middle-region type m1 was more frequent in H. pylori strains with genotype s1, while m2 alleles in most cases were detected in vacA s2 strains (Table 3). The iceA1 genotype was found in 35 (43.2%) of the 81 Lithuanian strains, and iceA2 genotype was detected in 40 (49.4%) of the cultures. Specific iceA subtypes were not revealed in 6 (7.4%) H. pylori strains tested by PCR.

While estimating relationship between potentially virulent H. pylori strains and clinical outcomes, significant differences (P<0.01) were found between isolates from DU and CG patients (Table 4). H. pylori strains cultured from DU patients more frequently (P<0.01) contained cagPAI and vacA s1 genotype (75.7% and 75.7%, respectively) in comparison to isolates from CG patients (45.5% and 40.9%, respectively). Evaluation of nucleotide sequence of the vacA middle-region revealed that vacA s2/m2 subtype was more frequent in CG than in DU patients (56.8% and 24.3%, respectively; P<0.05). We have not found any differences in the frequency of iceA1 genotype between the groups (Table 4).

Discussion

The clinical relevance of putative virulence-associated genes of H. pylori is still a matter of controversy. The present study provides data on distribution of H. pylori cagPAI status, vacA and iceA genotypes in Lithuanian patients with confirmed diagnoses of DU and CG. One-half to two-thirds of European and U.S. H. pylori strains carry the cag pathogenicity island, a 40-kb DNA segment; many of whose genes seem to help induce interleukin-8 secretion and, thereby, a strong and potentially damaging inflammatory

| vacA subtype | cagPAI type | P |
|--------------|------------|---|
|              | cagPAI+    | cagPAI-    |  |
| s1           | 93.8% (45/48) | 3% (1/33) | <0.001 |
| s2           | 6.2% (3/48)   | 97% (32/33) | <0.001 |

Table 2. Distribution of cagPAI and vacA s1/s2 genes in 81 Helicobacter pylori isolates from Lithuania

| Signal sequence type | Middle-region type |
|---------------------|--------------------|
|                     | m1     | m2     | m?     |
| s1                  | 23.9% (11/46) | 43.5% (20/46) | 32.6% (15/46) |
| s2                  | 0% (0/35) | 97.1% (34/35) | 2.9% (1/35) |
| P                   | <0.01   | <0.001 | <0.01 |

Table 3. Relationship between signal sequence (s1/s2) and middle-region typing of vacA gene for 81 Helicobacter pylori isolates from Lithuania

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response (23, 24). We ascertained that around two-thirds of Lithuanian H. pylori strains (59%) have cagPAI, and our data support previous reports from Western countries and suggest that persons colonized with cagPAI-positive H. pylori strains are at increased risk of developing peptic ulceration (24, 25).

The vacA gene is present in essentially all strains of H. pylori, but its nucleotide sequence varies among strains. Our study confirmed a strong association between the vacA genotype and cytotoxin phenotype of H. pylori strains. Our results are in agreement with other reports (6, 26) indicating a higher prevalence of the vacA s1 allele in patients with DU. Some previous studies (26, 27) suggested that the vacA m1 subtype of the vacA gene might be a suitable marker for the increased virulence of H. pylori. Our study has revealed that s2/m2 strains are more characteristic for chronic gastritis; however, we have not found that the H. pylori vacA s1/m1 subtype is associated with more severe outcome of H. pylori infection. The PCR-based typing system of the vacA middle-region failed to classify all Lithuanian strains. This unsuccessful PCR typing is probably due to mutations within primer regions and indicates diversity in the vacA middle-region sequence in different communities (28, 29).

Geographic differences have also been important in distribution of the vacA genotypes. The majority of H. pylori isolates from patients of Lithuania had the s1a subtype. This finding is in agreement with the earlier reports suggesting s1a predominance in Northern and Eastern Europe (30, 31). However, only two Lithuanian H. pylori strains showed vacA s1b genotype, and vacA s1c subtype was not detected, which is common in South Africa and East Asia, respectively, but infrequent in Europe (32, 33).

With regard to iceA gene, it was reported that iceA1 allele was related to the peptic ulcer disease in the United States (34), and the Netherlands (5); however, this finding has not been confirmed in other countries, such as Japan (16, 33), India (35), and Korea (16). Our study has not revealed an association between the iceA genotype and more severe disease outcome in Lithuanian patients. As reported earlier, H. pylori should not be considered as a single infectious organism, but as a worldwide population of bacterial variants, which might have different clinical impact in different parts of the globe (8).

**Conclusion**

Results of our study suggest that cagPAI and vacA s1 genotypes are associated with peptic ulceration in Lithuanian patients infected by Helicobacter pylori.
Helicobacter pylori lietuviškų padermių genotipai sergiensioms lėtiniu gastritu ir dvylikapirštės žarnos opalige

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Raktąžodžiai: Helicobacter pylori, dvylikapirštės žarnos opaligė, lėtinis gastritas, virulentiškumo veiksnių, cagPAI, vacA, iceA.

Santrauka. Darbo tikslas. Helicobacter pylori (H. pylori) infekcijos sukeltų ligų pobūdis gali būti susijęs su šios bakterijos padermių virulentiškumu. Įvairiuosiose geografiniose regionuose H. pylori padėmės papišimas skiriasi. Šio tyrimo tikslas – nustatyti H. pylori padėmės genetines charakteristikas (cagPAI, vacA, iceA genus) dvylikapirštės žarnos opaligė ir lėtiniu gastrititu sergiemams ligoniams iš Lietuvos.

Tyrimų kontingentas ir tyrimo metodai. Išverti 37 dvylikapirštės žarnos opaligė ir 44 lėtiniu gastritu (funkcine dispesija) sirgę ligoniai. H. pylori padėmėms, išaugintoms iš endoskopinio tyrimo metu paimtoms skrandžio kleštinės biopsinės medžiagos, cagPAI, vacA (s ir m potipiai) ir iceA genai buvo tiriama polimerazų grandinės reakcijos metodu, naudojant specifinius pradinės.

Rezultatai. Tyrimų rezultatai rodo, kad 59,3 proc. H. pylori padėmės turėjo seritotoksinį susijusį geną (cagPAI). Iš opalige sergančių pacientų 75,7 proc. buvo infekuoti cagPAI turinčiomis H. pylori padėmėmis, o tarp sergančių lėtiniu gastritu tokių genių buvo tik 45,5 proc. (p<0,01). Visoms tirtoms 81 H. pylori padėmėms radame vacA geną. Tarp sergančių padėmės 75,7 proc. buvo infekuoti vacA s1 genotipo padėmėmis, o sergančios lėtiniu gastrititu – 40,9 proc. (p<0,01). Tarp sergančių lėtiniu gastritu skirtingai nei opalige dažnesnė buvo vacA s2/m2 genotipo H. pylori padėmės (58,6 proc. ir 24,3 proc., p<0,05). Padermių, turinčių iceA1 genotipą, dažnis tiriama grupėse nesiskyrė (46,0 proc. ir 40,9 proc., p>0,05).

Išvada. H. pylori infekuotiemis ligoniams šios bakterijos cagPAI ir vacA s1 genotipų padėmės yra glaudžiai susijusios su dvylikapirštės žarnos opalige.

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