BACTÉRIOLOGIE

Prevalence of Helicobacter pylori vacuolating cytotoxin and its allelic mosaicism as a predictive marker for Iranian dyspeptic patients.

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Résumé : **Prévalence de cytotoxine vacuolisante d'Helicobacter pylori et son mosaïcisme allélique comme marqueur de dépistage chez des patients iraniens atteints de dyspepsie.**

L'infection par Helicobacter pylori touche la majorité de la population dans les pays en voie de développement. Cependant, le taux de complications gastro-intestinales telles que les ulcères ronds et différentes formes de malignité gastrique ne reflète pas celui de l'infection elle-même. Afin de déterminer si la cytotoxine (vacA) et son polymorphisme allélique peuvent servir de marqueurs de dépistage auprès de la population, nous avons isolé des souches de H. pylori chez 132 patients atteints de dyspepsie. L'ADN génomique H. pylori fut extrait et soumis à l'amplification PCR pour les allèles de cytotoxine. Le génotypage du gène vacA a permis d'identifier 68 % (70 sur 103) des patients atteints d'une dyspepsie non ulcéreuse et 79 % (23 sur 29) des patients avec un ulcère rond possédant le génotype s1. Le nombre de souches s1 était nettement plus élevé chez les patients avec ulcère comparés à ceux sans ulcère ($p < 0,05$). Sinon, 55 % des isolats des patients correspondaient au génotype m2 avec aucune corrélation pathologique. Le génotype s1m2 était le plus prévalent parmi les patients dans leur ensemble et était fortement corrélé avec le groupe à ulcère ($p < 0,05$).

dyspepsia
Helicobacter pylori
marker
Iran
Moyen-Orient

Summary:

Helicobacter pylori infects the majority of the population in the developing countries. However, the rate of gastrointestinal complications such as peptic ulcers and gastric malignancies has no parallel with the infection. In order to determine whether cytotoxin (vacA) and its allelic polymorphism can serve as screening markers for such a population, H.pylori strains were isolated from one hundred and thirty two dyspeptic patients. H.pylori genomic DNA was extracted and underwent PCR-amplification for the cytotoxin alleles. Genotyping of the signal sequence region of the vacA gene identified 68% (70 out of 103) of patients with non ulcer dyspepsia (NUD) and 79% (23 out of 29) of the patients with peptic ulcer disease (PUD) possessing the s1 genotype. S1 strains were significantly more prevalent among patients with PUD as compared to the NUD ($p < 0.05$). In regard to the middle region, 55% of the patient isolates belonged to the m2 genotype with no correlation to disease. The s1m2 genotype was the most prevalent among all patients and significantly correlated with the PUD group ($p < 0.05$)

dyspepsie
Helicobacter pylori
marqueur
Iran
Middle South Asia

Introduction

Helicobacter pylori is a gastric pathogen which infects half of the world population and causes antral gastritis, duodenal ulcers (7) and enhances the risk of gastric malignancies (10). Despite the high rate of *H. pylori* infection, only a small fraction of infected subjects goes beyond development of gastritis and develops peptic ulcers or gastric malignancies. In the developing countries, *H. pylori* infects the majority of the populations and the Iranian population possesses over 80% rate of infection (8). Antibiotic treatment of the majority of the population in the developing countries, in an effort to prevent or cure *H. pylori*-associated complications is, however, practically impossible. It is, therefore, absolutely essential to identify virulence factors which can serve as predictive markers for such populations. The genotyping protocol by ATHERTON *et al.* (1) for the vacuolating cytotoxin gene, *vac*A, of *H. pylori*, and its association with disease (3) has urged scientists to screen *H. pylori* isolates from different dyspeptic populations and correlate their genotype with the clinical diagnosis. For various reasons, which include the vast heterogeneity of *H. pylori* strains, the above association was not consistent for highly infected populations in countries such as China (9), Korea (15), and Japan (14). Due to the identification of strains untypable for the mid region by the originally described primers (1), ATHERTON *et al.* (2) described a new strategy in which a different pair of primers was used and was able to determine the hidden genotypes. In the present study, we

aimed at using this latter protocol on *H. pylori* strains isolated from the Iranian population in order to characterize local strains as well as to evaluate the *vac*A genotype markers for patient screening and prognosis purposes.

Materials and methods

Patients

One hundred and thirty two consenting patients suffering from gastrointestinal symptoms who referred to the endoscopy unit of two major hospitals in Tehran were enrolled in this study. The group was composed of 29 patients with peptic ulcer disease (PUD) and 103 patients with non ulcer dyspepsia (NUD). Fifty one percent of patients (67/132) were male and forty nine percent (65/132) were female with an age range of 37.6 +/- 16 all living in Tehran with low to middle class incomes. Gastric biopsies were obtained from the antrum and the corpus of the stomach and placed in urea transfer broth. Once the results of urease test were documented, biopsies were smeared on blood-agar plates (5% sheep blood/Brucella agar) and incubated under microaerobic conditions (37°C, 7% CO2) for 5-7 days. Grown bacteria underwent identity confirmation via specific microbiological tests including wet mount, urease, catalase and oxidase test.

Polymerase Chain Reaction

Bacterial genomic DNA was extracted by crude DNA extraction (13). The purified bacterial DNA underwent PCR amplification using primer pairs (table I) specific for the signal sequence and middle region of the *vac*A gene identifying s1, s2, m1 and m2 subtypes with the expected fragment sizes of 259 bp, 286 bp, 567 bp and 642 bp respectively (1, 2). PCR conditions were set up as instructed in the literature for each primer set (1, 2). Nontypable signal sequence and middle region were referred to as s0 and m0 (5).

Table I.

Sequence of PCR primers.
Séquence des amorces de PCR.

region	sequence of primers	location
signal sequence	F: 5' ATG GAA ATA CAA ACA CAC 3'	1-21
	R: 5' CTG CTT GAA TGC GCC AAA C 3'	241-259
middle region	F: 5' CAA TCT GTC CAA TCA AGC GAG 3'	1287-1307
	R: 5' GCG TCA AAA TAA TTC CAA GG 3'	1912-1931

Statistical Evaluation

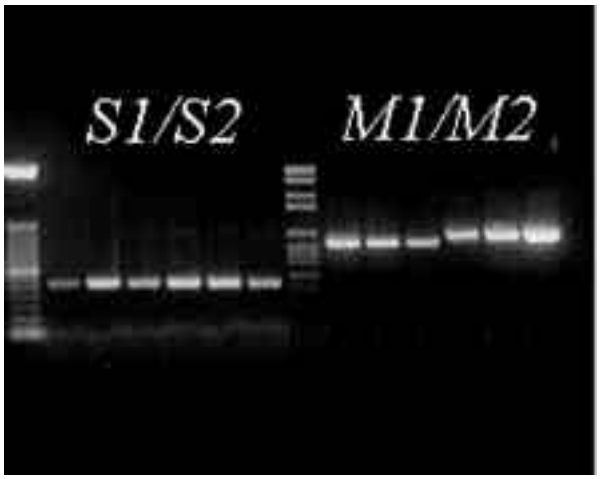
Correlation analysis between laboratory findings and clinical manifestations of disease was performed by χ^2 analysis and Fisher's exact test using Statview™ software.

Results and discussion

A garose gel electrophoresis visualizing the PCR products of the signal sequence and middle region of various strains is demonstrated in figure 1. The distribution of the signal sequences and the middle regions in relation to the two clinical groups for the 132 *H. pylori* strains are shown in table 1. It can be seen that 68% (70 out of 103) of the NUD group belonged to the s1 genotype and 79% (23 out of 29) of the PUD group possessed this genotype. The prevalence of s1 in the PUD group was significantly higher than in the NUD group (79% *versus* 68%, $p < 0.05$). This demonstrates that among Iranian *H.pylori* strains, the *vac*A s1 genotype is the most prevalent and is associated with peptic ulcer disease. These results are in accordance with previous findings by ATHERTON *et al.* (3).

Figure 1.

PCR amplification of signal sequence and middle region of the vacA gene in H. pylori DNA extracts. Lanes 2-4 & 9-11:s1m1 strains, lanes 5-7 & 12-14:s1m2 strains, lanes 1 & 8:DNA markers.
Amplification PCR de la sequence signal et de la région médiane du gène vacA dans les extraits ADN de H. pylori. Lignes 2-4 & 9-11: souches s1m1, lignes 5-7 &12-14:souches s1m2, lignes 1&8: marqueurs ADN.



All of the depicted strains are of s1 genotype.
Toutes les souches décrites appartiennent au genotype s1.

Furthermore, 55% of the strains possess the *vac*A m2 genotype, as shown in table I. However, the middle region typing showed no significant correlation with the clinical status of the patients. This finding has been also reported from Germany (11) where the majority of strains possessed the m2 genotype with no correlation with the clinical manifestations of disease. In a study from Poland, it has been reported that a significant number of *H. pylori* strains remains unclassified for the middle region (5), and Asian strains were also reported to differ in gene sequence (2). Therefore, a new strategy was described by ATHERTON *et al.* for strains untypable for the middle region (2), and using this strategy all previously untypable *H. pylori* strains were found to be *vac*A m1, except for one m1/m2 hybrid (2). In spite of using this new strategy, we detected two percent of the strains being untypable for the signal region and twelve percent for the middle region. The presence of a significant number of untypable strains further indicates the extensive heterogeneity among *H. pylori* strains from different geographic regions. The untypable strains should be sequenced to determine the exact location of variation among these strains.

Table II.

Distribution of vacA genotypes among patients with peptic ulcer disease and non-ulcer dyspepsia.
Distribution des génotypes vacA chez les patients atteints d'ulcère peptique et de dyspepsie non ulcéreuse.

region amplified	NUD		PUD		total	
	nb patients	%	nb patients	%	nb patients	%
s1	70	68	23	79	93	71
s2	32	31	4	14	36	27
s0	1	1	2	7	3	2
m1	34	33	8	27	42	33
m2	57	55	17	59	74	55
m0	12	12	4	14	16	12
s1/m1	29	28	6	21	35	27
s1/m2	29	28	14	49	43	33
s2/m2	28	27	3	10	31	23
s2/m1	4	4	0	0	4	3
s1/m0	12	12	3	10	15	11
s2/m0	0	0	1	3	1	1
s0/m1	1	1	2	7	3	2

The collective assessment of the *vac*A genotype signal sequence and middle region determines that the significant majority of the PUD group consists of the s1m2 genotype ($p < 0.05$) and not the s1m1 genotype as widely found in previous reports (3). In the NUD group, however, the three *vac*A s1m1, s1m2, and s2m2 genotypes were equally distributed. The *vac*A s2m1 genotype was not represented in the PUD group but was found in four isolates from the NUD group, in agreement with a report from Africa in which *vac*A s2m1 strains were clearly characterized (6).

The vast heterogeneity between *H. pylori* strains (12) and also worldwide geographic variations (4) can explain the differences found in the different *vac*A genotyping reports. The high frequency of *H. pylori* infection worldwide and the chronic persistence of bacteria in the host have provided grounds for extensive genetic variations, possibly in order to guarantee survival of the bacteria. There are added grounds for this heterogeneity in highly infected countries like Iran, where the majority of the population is infected (8). High rates of infection have given chance to multiple strain infection, and increasing the chance of exchange of genetic material between strains as well as selection for specific strains. In an effort to survive, *H. pylori* strains in developing countries may have been selected for changes that would reduce virulence and therefore inactive virulent genes could have been positively selected resulting in a more benign infection. This theory may explain the high prevalence of s1 strains (otherwise known as the more virulent strains) among the NUD group of Iranian dyspeptic patients.

Conclusion

This study showed that s1 and s2 were markers of the *vac*A gene that differentiated between NUD and PUD groups of Iranian dyspeptic patients, whereas the m1 and m2 markers did not give any additional differentiation between these two groups. Nevertheless, the s1m2 genotype is the most prevalent genotype among the PUD group of patients. Thus, the s1 region can be used as a screening measure for identifying "high risk" patients who call for treatment and closer follow-up among the *H. pylori*-infected dyspeptic patients. In addition a significant number of strains was untypable by the new strategy and should therefore be characterized further via sequence specific primers.

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