Relationship between *Helicobacter pylori vacA* genotypes status and risk of peptic ulcer in Saudi patients

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ABSTRACT

Objectives: To determine if there is a significant correlation between different *Helicobacter pylori* (*H. pylori*) *vacA* genotypes strains and severe gastric clinical outcomes.

Methods: A total of 1104 gastric biopsies from 368 patients who presented with symptoms suggestive of chronic gastritis or peptic ulcer were taken from the main hospitals in the western region of Saudi Arabia from July 2004 to July 2005. These samples were cultured for *H. pylori*, and a polymerase chain reaction (PCR) was carried out to determine vacA genotypes status.

Results: One hundred and three (28%) patients were positive for *H. pylori* using culture technique. The distribution of *vacA* genotypes was 13 for *vacAs1m1*, 47 for *vacAs1m2* and 43 for *vacAs2m2*. None of the clinical isolates were *vacAs2m1* positive. The study showed a significant correlation between the *vacAs1m2* genotype and gastritis cases, and a significant correlation between *vacAs1m1* genotype and ulcer cases.

Conclusion: The results of this study might be used for the identification of high-risk patients who are infected by *vacAs1m1* genotype *H. pylori* strains.

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Helicobacter pylori (*H. pylori*) is a gram-negative curved rod with a tuft of 4-7 polar flagella. The organism inhabits the gastric mucosa of the human stomach in approximately half of the world's population for a lifetime.[1-3] *Helicobacter pylori* induces gastric mucosal inflammation, causing gastritis which may progress into peptic ulcers.[1,4,5] *Helicobacter pylori* is the major environmental factor in the development of gastric cancer increasing from 4-6 folds the risk of its development.[6-9] One of the possibilities to explain the variation in clinical outcome caused by infection with *H. pylori* is that a considerable genetic variation exists between different strains of *H. pylori*.[10-12] *Helicobacter pylori* strains that having variant genes can causing a considerable inflammatory response in the host more severely than other strains.[10-12] *VacA*, the gene encoding the vacuolating cytotoxin, has a mosaic structure consisting of one of 3 signal sequence types (s1a, s1b, s2), and one of 2 mid-region types (m1 and m2).[13,14] Type s1 strains were significantly more common than type s2 strains in patients with a past or present history of ulcer.[15-18] The *vacA* genotype of strain is closely associated with cagA gene to induce peptic ulceration.[15,18,19] All *vacA* positive isolates included were identified among the subjects with *H. pylori* associated gastritis.[18,19] Type

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s1m1 and s1m2 strains produce high and moderate level of toxin. Whereas the s2m2 strain produces little or no toxin.[10,19,20] The aim of this study was to determine the most common *vacA* genotype among *H. pylori* isolates from a group of Saudi patients with gastric complaints using polymerase chain reaction (PCR) as a typing system.

Methods. ***Patients' samples.*** From July 2004 to July 2005, 1104 gastric biopsies from 368 patients who presented with symptoms suggestive of chronic gastritis or peptic ulcer were taken from gastric antrum and corpus from the main hospitals in the western region of Saudi Arabia (Al-Noor Specialist Hospital [560 bed], King Abdul-Aziz Hospital [272 bed], General King Fahad Hospital [710 bed], King Abdul-Aziz Hospital and Oncology Centre [425 bed] and King Faisal Hospital [221 bed]). Patients were excluded if they had a history of previous *H. pylori* treatment.

Gastric biopsies transportation and culture. Gastric biopsies were transported in a 0.5 ml Brucella broth media (Oxoid, UK). Three Gastric biopsies were obtained from each patient, one was used for rapid CLO test (for determination of urease activity) (Oxoid, UK) and the others were cultured on *H. pylori* selective agar (Oxoid, UK) and incubated at 37°C in a BBL GasPak (Becton-Dickinson, USA) containing a Campy-Pak Plus microaerophilic system generator (Becton-Dickinson, USA) and incubated for 7 days. The identity of *H. pylori* clinical isolates were confirmed by colonial morphology, Gram-stain (curved Gram-negative bacilli) and positive reaction for oxidase, catalase and urease tests.

Deoxyribonucleic acid methods. Chromosomal staphylococcal DNA was extracted and purified according to previous descriptions.[21] Primers were designed to amplify 259bp, 286bp, 570bp and 645bp products within the *vacAs1, vacAs2, vacAm1* and *vacAm2*, and 203bp product within the Ure gene (positive control) (**Table 1**). A 5 μl of each PCR primer (0.025 μM final concentration; TIB Molbiol, Germany; **Table 1**) plus 5 μl of the extracted DNA were added to a PCR master mix (100 mM Tris-HCl, and 500 mM KCl at pH 8.3 at 20°C, 1.5 mM MgCl2, 200 μM each deoxyribonucleoside triphosphate, 0.025U Taq polymerase [Qiagen, UK]). This mixture was then heated to 94^0C for 5 minutes and then subjected to 35 cycles each of 95^0C for one minute, 52^0C for one minute, and 72^0C for one minute with a final extension of 72^0C for 7 minutes using the Perkin Elmer Geneamp PCR system 2400. A 10 μl aliquot of each PCR product was loaded onto 1% agarose (Sigma, USA) and run at 90V for one hour prior to viewing under UVP BioDoct-It digital imaging system (UVP, Inc, Cambridge, UK) to determine *vacA* genotypes.

Statistical analysis. Distribution of *vacA* genotypes among clinical isolates, and its correlations with endoscopic findings were recorded and analyzed using SPSS (version 10). Chi-squared test was used to compare genotype frequencies using SPSS (version 10).

Results. Among the 368 suspected patients to be infected with *H. pylori* by means of clinical features and endoscopic findings, 103 (28%) were positive using culture technique. Among these positive cases, PCR distribution of *vacA* genotypes were 13 (12.6%) for *vacAs1m1*, 47 (45.6%) for *vacAs1m2*, and 43 (41.8%) for *vacAs2m2*. None of the clinical isolates was *vacAs2m1* positive. The results revealed that all 103 cases were positive for *Ure* gene. The study showed that positive samples from gastric ulcer, gastritis, normal cases according to endoscopic findings were correlated with 6.6%, 70.8%, and 22.6% with the presence of *H. pylori* (**Table 2**). The results showed a high percentage of *vacAs1m2* with a distribution of 56.8% in gastritis cases (p=0.0001). In case of ulcer, the highest rates were among *vacAs1m1* with a frequency of 71.4% (p=0.001), while in normal cases the highest rates were among *vacAs2m2* with a percentage of 86.4%.

Discussion. *Helicobacter pylori* is a microaerophilic, gram-negative bacterium that colonizes the gastric mucosa of approximately 50% of the world's population, and is a primary pathogenic factor in benign and malignant gastroduodenal disease.[1-9] Recent studies have shown that different *vacA* genotypes can lead to different clinical outcome consequences in certain populations.[13-20] In this study, 28% of patients tested were *H. pylori* positive. This finding is much less than what has been reported elsewhere in the Kingdom with rates including 87% in Eastern, 61.6% in Central and 85% in Western regions. This variability in the incidence rates between different studies might be attributable to: 1) the differences in the methods used for identification this organism, 2) different demographic distribution of the bacteria among various regions, and 3) previous antibiotic consumption.[24] These reasons might also explain the different distribution of this organism among different international studies.[12,24,25] The *vacA s1m1, s1m2* and *s2m2* genotypes were detected in all 103 *H. pylori* samples PCR tested. In this study, the prevalence of the *vacA* genotypes *s1m1* was detected

Table 1 - Properties of oligonucleotides primers bp - base pair, F - forward, R - reverse.

Primer designation	Target gene	Nucleotide sequence, 5' to 3'	Amplicon size (bp)	Reference
Ure1	Urease1	TAA CAA ACC GAT AAT GGC GC	203	22
Ure2	Urease2	CAT CTT GTT AGA GGG ATT GG		
*vacA s1*F	Vacuolating CytotoxinAS1F	ATG GAA ATA CAA CAA ACA CAC	259	23
*vacA s*1R	Vacuolating CytotoxinAS1 R	CTG CTT GAA TGCGCC AAA C		
*vacA s2*F	Vacuolating CytotoxinAS2F	ATG GAA ATA CAA CAA ACA CAC	286	23
*vacA S2*R	Vacuolating CytotoxinAS2R	CTG CTT GAA TGC GCC AAA C		
*vacA m1*F	Vacuolating CytotoxinAm1F	CAA TCT GTC CAA TCA AGC GAG	570	23
*vacA m1*R	Vacuolating CytotoxinAm1R	GCG TCT AAA TAA TTC CAA GG		
*vacA m2*F	Vacuolating CytotoxinAm2F	CAA TCT GTC CAA TCA AGC GAG	645	23
*vacA m2*R	Vacuolating CytotoxinAm2R	GCG TCT AAA TAA TTC CAA GG		

Table 2 - *VacA* genotype frequencies according to endoscopic findings.

Endoscopic findings	No. of *vacA* genotypes (%)			
	vacAs1m1	*vacAs1m2*	*vacAs2m1*	*vacAs2m2*
Gastritis	8 (10.8)	42 (56.8)	0 (0)	24 (32.4)
Ulcer	5 (71.4)	2 (28.6)	0 (0)	0
Normal	0	3 (13.6)	0 (0)	19 (86.4)

in 12.6%, *s1m2* in 45.6% and *s2m2* in 41.8%. No single case for *vacAs2m1* genotype was detected in this study. This finding is in agreement with previous studies as this genotype was reported to be rare.[26,27] In this study, the most pathogenic *vacA* genotype which is *vacAs1m1* was present in 71% of sampled studied, these are in agreement with previous studies where they found association between this genotype and severe gastric outcomes.[15-20] In addition, this genotype was not detected in individuals with normal endoscopic finding. These findings support the role of *vacAs1m1* genotype in severe clinical outcomes.[15-18]

In conclusion, the results of this study might be used for the identification of high-risk patients who are infected by *vacAs1m1* genotype *H. pylori* strains. These patients infected with such strains should have more tension regarding anti-Helicobacter treatment to prevent reoccurrence and prevent severe clinical outcome such as peptic ulcer and gastric carcinoma later on in their life.

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References

1. Dunn B, Cohen H, Blasser M. *Helicobacter pylori*. ***Clin Microbiol Rev*** 1997; 10: 720-741.
2. Vaira D, Holton J, Ricci C, Menegatti M, Gatta L, Berardi S, et al. Review article: the transmission of *Helicobacter pylori* from stomach to stomach. ***Aliment Pharmacol Ther*** 2001; 15: 33-42.
3. Aguilar G, Ayala G, Fierros-Zarate G. *Helicobacter pylori*: Recent Advances in the study of its Pathogenicity and Prevention. ***Salud Publica Mexico*** 2001; 33: 237-247.
4. Penta R, De Falco M, Iaquinto G, De Luca A. *Helicobacter pylori* and gastric epithelial cells: from gastritis to cancer. ***J Exp Clin Cancer Res*** 2005; 24: 337-345.
5. Yamazaki S, Yamakawa A, Okuda T, Ohtani M, Suto H, Ito Y, et al. Distinct diversity of vacA, cagA, and cagE genes of *Helicobacter pylori* associated with peptic ulcer in Japan. ***J Clin Microbiol*** 2005; 43: 3906-3916.
6. Warren J, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. ***Lancet*** 1983; I: 1273-1275.

7. Blaser M, Parsonnet J. Parasitism by the slow bacterium *Helicobacter pylori* leads to altered gastric homeostasis and neoplasia. ***J Clin Invest*** 1994; 94: 4-8.
8. Kupiers E. Review article: exploring the link between *Helicobacter pylori* and gastric cancer. ***Aliment Pharmaco Ther*** 1999; 13: 3-11.
9. Zagari RM, Bazzoli F. Gastric cancer: who is at risk? ***Dig Dis*** 2004; 22: 302-305.
10. Axon A. Are all Helicobacters equal? Mechanisms of gastroduodenal pathology and their clinical implications. ***Gut*** 1999; 45: 11-14.
11. Figueiredo C, Machado JC, Yamaoka Y. Pathogenesis of *Helicobacter pylori* Infection. ***Helicobacter*** 2005; 10: 14-20.
12. Kauser F, Hussain MA, Ahmed I, Srinivas S, Devi SM, Majeed AA, et al. Comparative genomics of *Helicobacter pylori* isolates recovered from ulcer disease patients in England. ***BMC Microbiol*** 2005; 5: 32-??.
13. Cover T, Blaser M. H. pylori: a Bacterial Cause of Gastritis Peptic Ulcer Disease and Gastric Cancer. ***ASM News*** 1995; 5: 21-26.
14. Panchal PC, Forman JS, Blumberg DR, Wilson KT. *Helicobacter pylori* infection: pathogenesis. ***Curr Opin Gastroenterol*** 2003; 19: 1-3.
15. Aydin F, Kaklikkaya N, Ozgur O, Cubukcu K, Kilic AO, Tosun I, et al. Distribution of vacA alleles and cagA status of *Helicobacter pylori* in peptic ulcer disease and non-ulcer dyspepsia. ***Clin Microbiol Infect*** 2004; 10: 1102-1104.
16. Gatti LL, Fagundes EK, Leite KR, Bastos EL, Vicentini LR, Silva LC, et al. cagA vacA alleles and babA2 genotypes of *Helicobacter pylori* associated with gastric disease in Brazilian adult patients. ***Diagn Microbiol Infect Dis*** 2005; 51: 231-235.
17. Wu CC, Chou PY, Hu CT, Liu ZC, Lin CY, Tseng YH, et al. Clinical Relevance of the vacA, iceA, cagA, and flaA genes of *Helicobacter pylori* strains isolated in Eastern Taiwan. ***J Clin Microbiol*** 2005; 43: 2913-2915.
18. Siavoshi F, Malekzadeh R, Daneshmand M, Ashktorab H. *Helicobacter pylori* endemic and gastric disease. ***Dig Dis Sci*** 2005; 50: 2075-2080.
19. Tiveljung A, Borch K, Jonasson J, Mardh S, Petersson F, Monstein H. Identification of *H. pylori* in Gastric Biopsies by PCR Based on 16S rDNA Sequences: a Matter of Little Significance for the Predication of *H. pylori*-Associated Gastritis? ***J Med Microbiol*** 1998; 10: 659-704.
20. Doorn L, Figueiredo C, Megraud F, Pena A, Midolo P, Quint D, et al. Geographic distribution of vacA allelic types of *Helicobacter pylori*. ***Gastroenterology*** 1999; 12: 823-830.
21. Pitcher DJ, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidinium thiocyanate. ***Lett Appl Microbiol*** 1989; 8: 151-156.
22. Persing D, Smith T, Tenover F, White T. Diagnostic Molecular Microbiology Principles and Applications. Washington (DC): Rochester Foundation; 1993. p. 282-287.
23. Doorn L, Figueiredo C, Rossau R, Jannes G, Asbroec M, Sousa J, et al. Typing of *Helicobacter pylori* vacA Gene and Detection of cagA gene by PCR and Reverse Hybridization. ***J Clin Microbiol*** 1998; 23: 1271-1272.
24. Bazzoli M, Al-Qurain A. *Campylobacter pylori* in Saudi Arabia under upper gastrointestinal endoscopy. ***Saudi Med J*** 1999; 7: 516-518.
25. Duck M, Wang Y. Stool Antigen Assay can effectively screen *Helicobacter pylori* infection. ***Gastroenterology*** 2001; 6: 98-103.
26. Letley D, Lastovica A, Louw J, Hawkey C, Antherton J. Allelic diversity of the *Helicobacter pylori* vacuolating cytotoxin gene in South Africa: rarity of the vacA s1a genotype and natural occurrence of an s2/m1 allele. ***J Clin Microbiol*** 1999; 7: 1203-1205.
27. Martinez A, Gonzalez F, Kawaguchi F, Montya R, Corvala A, Madariaga J, et al. *Helicobacter pylori*: cagA analysis and vacA genotyping in Chile. Detection of a s2/m1 strain. ***Rev Med Chi*** 2001; 11: 1147-1153.