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SHORT COMMUNICATION

The Relationship between *H. pylori* Virulence Genotypes and Gastric Diseases

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Abstract

There have been no reports on the relationship between virulence genes and gastric diseases based on the same bacterial colonization density. Our results indicated that *Helicobacter pylori* virulence genes were more relevant than colonization density as a pathogenic mechanism of gastric diseases, which helps elucidate the pathogenic mechanisms of bacteria and aids in the development of improved strategies for the treatment of gastric disease.

Key words: *H. pylori,* colonization density, *cagA*, *vacA*, *iceA*

Approximately half of the world's population is infected with *Helicobacter pylori* and it has been associated with chronic gastritis, peptic ulcer and gastric carcinoma (Momtaz *et al*., 2010). Most infected people remain asymptomatic, and only 15–20% of *H. pylori* positive individuals develop the associated diseases (Franco *et al*., 2008). Why some infected people develop these sequelae and others do not is unknown, but one possible explanation is that some *H. pylori* strains are more pathogenic than others. Over the last few years, increased attention has been given to the significance of *H. pylori* virulence genes, such as the vacuolating cytotoxin (*vacA*), the cytotoxin associated gene A(*cagA*) and a gene induced by contact with the gastric epithelium (*iceA*)(Boyanova *et al*., 2009). Nevertheless, the clinical relevance of the virulence associated genes of *H. pylori* is still a matter of controversy. Several studies have reported an influence of virulence genes on the clinical outcomes of *H. pylori* infections in different geographical regions. One hypothesis for why these strains are associated with different clinical outcomes is that there is a marked discrepancy between the number of individuals colonized and those with clinical symptoms. Low bacterial colonization density was correlated significantly with mild degrees of gastric neutrophil infiltration (Kaklikkaya *et al*., 2006) and macroscopic erosions (Molnar *et al*., 2008), and high bacterial density was more significantly associated with peptic ulcers than chronic gastritis (Boyanova, 2007). Therefore, quantified bacterial density is optimal for helping elucidate the pathogenic mechanisms of *H. pylori* and aiding in the development of improved strategies for the treatment of gastric disease.

The aim of our study was to explore the relationship between virulence-associated genes and gastric diseases based on the same bacterial colonization density.

This study was approved by the Ethics Committee of the China Medical University and all subjects signed an informed consent form before inclusion. A total of 174 patients (99 males and 75 females, 30–82 y, mean age 53 y) were involved in the study. Three biopsy specimens from each patient were placed in 10% formalin and processed in paraffin blocks. All sections were stained with hematoxylin and eosin and a single experienced pathologist reviewed all the slides according to the criteria proposed in the updated Sydney system. The distribution of clinical disease was as follows: 67 patients had superficial gastritis (SG); 20 patients had gastric ulcer (GU); 63 patients) had atrophic gastritis (AG) and 24 patients had gastric cancer (GC).

Detection of *H. pylori* was carried out by immunohistochemical staining using polyclonal anti-*H. pylori* antibody and peroxidase-conjungated streptavidin (*DAKO A/S, Denmark*). We graded the density of

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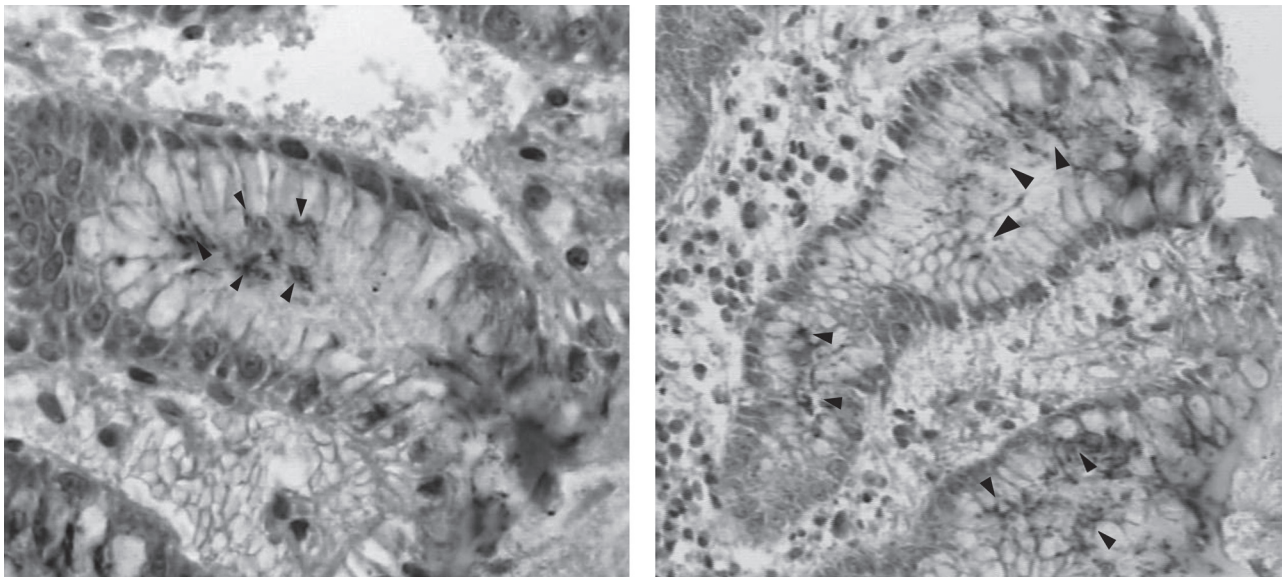

Fig. 1. Representative gastric sections stained immunohistochemically for anti-*H. pylori*-IgG. H pylori(arrow) were stained brown and were demonstrated on the apical and/or lateral surface of the surface mucous cells on which *H. pylori* were seen as small aggregates with detached epithelial cells.

A: low bacterial density (original magnification ×200); B: moderate to severe bacterial density (original magnification ×100)

H. pylori infection according to the number of individual bacteria that were counted in a highly magnified visual field (×1000 light-microscopy) (Fig. 1). The density of *H. pylori* infection was defined as follows: 0 = 0; 1 + = 1 – 9; 2 + = 10 – 29; and 3 + = 30 – 99. Details of the method have been published elsewhere (Tokunaga *et al*., 2000).

In this study, we extracted *H. pylori*-DNA directly from gastric tissues infected by *H. pylori*. PCR amplifications (Table I) were performed in an automated thermal cycler. PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide staining and were visualized under a short wavelength ultraviolet light source. The results were analyzed using the x2 test and with Yates continuity correction and the Fisher exact test. Results were considered statistically significant when the *p*-values were less than 0.05.

The results showed that patients suffering from SG, GU, AG and GC were predominantly affected by moderate to severe bacterial infection. Bacterial colonization density was moderate to severe (grades 2–3) in 53/67 of SG strains and 47/63 of AG strains, showing there was no significant correlation between the density of the bacterial colonization and the presence

Table I
Polymerase chain reaction for amplification of *cagA*, *vacA* and *iceA* genes

Region	Prime	Nucleotide sequence(5'-3')	Size of product (bp)
cagA	CAGAF	GGCAATGGTGGTCCTGAGGCTAGGC	324
	CAGAR	GAGAATCTTTAATCTCAGTTCGG	
vacAs1/s2	VA1-F	ATGAGAATACAACAAACACAC	259/286
	VA1-R	CTGCTTAGATGCGCCAAAC	
vacA m1a	VA3-F	GGTCAAAATGCGGTCATGG	290
	VA3-R	CCATTGGTACCTGTAAGAAC	
vacAm1b	VAM-F3	GGCCCCAATGCAGTCATGAGT	291
	VAM-R3	GCTGTTAGTGCCTAAAAGAGCAT	
vacAm2	VA4-F	GAGGCCCCAGAGAACATTG	352
	VA4-R	CATAACTAGCGCCTTGCAC	
iceA1	ICEA1F	GTGTTTTTAACCAAAGTATC	247
	IVEA1R	CTATAGCCASTYTCTTTGCA	
iceA2	ICEA2F	GTTGGGTATATCACAATTTAT	334
	ICEA2R	TTRCCCTATTTTCTAGTAGGT	

Table II
Distribution of bacterial colonization density in patients

Gastric diseases	Bacterial colonization density		Total
	0–1	2–3	
SG	14 (20.90)	53 (79.10)	67
GU	3 (15.00)	17[a] (85.00)	20
AG	16 (25.40)	47[b] (74.60)	63
GC	9 (37.50)	15[c] (62.50)	24
Total	34	66	174

0–1,low bacterial density; 2–3, moderate to severe bacterial density;
[a] p = 0.7931vs SG; [b] p = 0.689vs SG; [c] p = 0.183vs SG

gastric diseases compared to the density of bacterial colonization, it should be recognized that most patients suffering from SG, AG and GC that were infected by bacteria were from areas with a higher prevalence of gastric cancer and where moderate to severe density of *H. pylori* was prevalent, which suggests that the high bacterial colonization density, to a lesser degree, may be a factor in the development of *H. pylori* induced gastric diseases. Many more cases need to be further evaluated to provide more reliable results about the relationship between specific genotypes and gastric diseases based on the same density of bacteria.

Table III
Distribution of bacterial genotypes in patients based on the same bacterial density (grade 2–3)

Diseases	*H. pylori* genotypes (%)						Total
	cagA	vacAs1	vacAm1	vacAm2	iceA1	iceA2	
SG	30 (56.60)	48 (90.57)	36 (67.92)	17 (32.08)	35 (66.04)	30 (56.60)	53
GU	10 (58.82)	17 (100.00)	10 (58.82)	12[d] (70.59)	10 (58.82)	11 (64.71)	17
AG	21 (44.68)	30 (63.83)	15[a] (31.91)	40[c] (85.11)	22 (46.81)	20 (42.55)	47
GC	7 (46.67)	11 (73.33)	3[b] (20.00)	7 (46.67)	4[e] (26.67)	7 (46.67)	15
Total	68 (51.52)	106 (80.30)	64 (48.48)	76 (57.58)	71 (53.79)	68 (51.52)	132

[a] P = 0.000vs SG; [b] P = 0.001vs SG; [c] P = 0.000vs SG; [d] P = 0.002vs SG; [e] P = 0.015vs SG

of atrophic gastritis ($p = 0.689$). In addition, bacterial density was moderate to severe (grades 2–3) in 53/67 of SG strains and 15/24 of GC strains, showing there was no significant correlation between the density of the bacterial colonization and GC ($p = 0.183$)(Table II). SG patients more often harbored strains with the *vacAm1* genotype (67.92%, 36 of 53 cases) than the AG or GC patients (31.91%, 15 of 47 cases, $p = 0.000$ and 20.00%, 3 of 15 cases, $p = 0.001$, respectively). In addition, AG patients more often harbored strains with the *vacAm2* genotype (85.11%, 40 of 47 cases) than the SG patients (32.08%, 17 of 53 cases, $p = 0.000$) (Table III).

In order to exclude interference from the density of bacteria, we explored the relationship between *H. pylori* virulence genes and gastric diseases based on the same colonization density. We first found the *vacAm2* strain was significantly associated with AG. Few have reported an association between *vacAm2* and gastric diseases except v*acAm2* strains were associated with gastritis in Iranian patients (Dabiri *et al.*, 2010). The reason why *vacAm* alleles induce different types of gastritis might be that the *m1* and *m2* forms of the *VacA* cytotoxin may recognize different receptors on human gastric epithelial cells (Nogueira *et al.*, 2001), and there is a higher level of cytotoxin production by *vacA s1*/*m1* strains as compared to *vacA s1*/*m2* strains. Although the results suggested virulence genes might play a critical role in

In conclusion, our results indicated that *H. pylori* virulence genes were more relevant than colonization density as a pathogenic mechanism of gastric diseases, which helps elucidate the pathogenic mechanisms of bacteria and aids in the development of improved strategies for the treatment of gastric disease.

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