Helicobacter pylori: correlation of the virulence marker *iceA* allele with clinical outcome in a high prevalence area

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ABSTRACT

The association of *Helicobacter pylori* virulence marker 'induced by contact with epithelium A' (*iceA*) allele types was determined in *H.pylori*-related diseases and virulence markers. Gastric biopsies were obtained at EGD from patients for culture, histopathology and polymerase chain reaction (PCR) for *iceA* types, *cagA* and *vacA* alleles. Two hundred and eighty-four *H. pylori* isolates were examined. *iceA* type1 was positive in 177 (62%) and *iceA* type 2 in 158 (56%). In iceA type 2, gastric ulcer was present in 34 (21%) ($P<0.001$) and carcinoma in 28 (25%) ($P=0.002$), compared to nine (8%) and 2 (2%) in *iceA* type 2-negative cases. For *iceA* type 2 , 139 (88%) were associated with chronic active gastritis compared to 95 (75%) ($P=0.006$) in *iceA* type 2-negative. *H. pylori cagA* was positive in 101 (64%) *iceA* type 2 strains compared to 57 (45%) in negative strains ($P=0.002$). *H. pylori iceA* type 2 was dominant and associated with *cagA*, chronic active inflammation, gastric ulcer and carcinoma.

KEY WORDS: Alleles.
Genes.
Helicobacter pylori.
Stomach neoplasms.
Stomach ulcer.

Introduction

Helicobacter pylori is a Gram-negative bacterium that colonises the human gastric mucosa. It is the major cause of chronic gastritis and plays an important role in the pathogenesis of peptic ulcer, gastric carcinoma and primary B-cell gastric lymphoma.[1] Clinical outcome has been associated with various host genetic, environmental and *H. pylori* virulence factors.[2]

The virulence factors that play an important role in *H. pylori* pathogenicity change with the geographic area. Genetic diversity among *H. pylori* strains helps to account for varying clinical outcomes among those who are colonised.[3] Candidate markers for distinguishing disease-associated *H. pylori* from less virulent strains include presence of the cag pathogenicity island, *vacA s1/m1* allele polymorphisms, and intact outer immunoprotein A (oipA).[4]

Earlier studies showed that *iceA* has two main allelic variants, *iceA* type 1 and *iceA* type 2.[5,6] The expression of *iceA* type 1 has been shown to be upregulated on contact between *H. pylori* and human epithelial cells, resulting in enhanced mucosal interleukin (IL)-8 expression and acute antral inflammation.[7,8] It has been reported before that the *iceA* allelic type is independent of the *cagA* and *vacA* status, and there is significant association between the presence of the *iceA* type 1 allele and peptic ulcer disease.[9] The *iceA* type 1 allele is reported to be predominant in Japan and Korea, and the *iceA* type 2 allele in the United States and Colombia.[6]

Pakistan is a developing country where *H. pylori* seropositivity increases with age and low-middle socioeconomic status.[5] *cagA* was found to be negative in the majority of non-ulcer dyspepsia patients with *H. pylori* infection. However, *cagA* was associated with peptic ulcer and gastric carcinoma.[10] *vacA* alleles *s1am1* and *s1bm1* were found to be associated with *H. pylori*-associated diseases and inflammation.[11]

iceA presence in all *H. pylori* strains has not been studied before in our patient population with dyspeptic symptoms. The current study, therefore, aims to characterise *iceA* types in *H. pylori*, their relationshp with *H.pylori*-related disease, histological change and virulence markers such as *cagA* and *vacA* alleles.

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Materials and methods

Patients

Two hundred and thirty-two patients were enrolled and three hundred and twenty-six isolates were obtained. There were 172 (74%) males and 60 (26%) females (mean age: 45±13, range:18–79 years). They attended the gastroenterology out-patient and endoscopy suite between September 2013 and August 2014. The study was approved by the institutional ethics review committee. All patients gave an informed consent for endoscopy and participation in the study.

Participants were diagnosed as follows: non-ulcer dyspepsia (NUD) in 188 (81%), gastric carcinoma (GC) in 15 (6%), duodenal ulcer (DU) in 13 (6%) and gastric ulcer (GU) in 16 (7%). Of the GC cases, 10 (67%) were in the corpus and five (33%) in the antrum. All were adenocarcinomas (eight [54%] diffuse, seven [46%] intestinal). None of the patients had received antibiotics, acid-reducing drugs (e.g., H2-receptor antagonists, acid pump inhibitors), non-steroidal anti-inflammatory drugs or bismuth compounds in the previous four weeks.

Clinical symptoms at the time of presentation and endoscopic findings were noted. Gastric biopsy specimens were taken from the antrum and corpus. Two biopsy specimens were removed each for the rapid urease test (Pronto Dry), histopathology and polymerase chain reaction (PCR). Specimens for histopathology were dispatched in formalin and for PCR in 0.9% normal saline. The PCR for *cagA* 5′ terminal, *cagA*-promoter region, *vacA* alleles (i.e., *s1a*, *s1b*, *s*2 and *m1*, *m2*, and *iceA* [type 1 and 2]) were analysed. *H. pylori* strains with single *iceA* type were analysed while those with dual *iceA* types were excluded.

Bacterial culture

The specimens were transported immediately in sterile normal saline to isolate *H. pylori*. Thus, within one hour of collection each specimen was homogenised in sterile Eppendorf tubes using an electric homogeniser. The resulting suspension was inoculated onto Columbia blood agar (Oxoid) medium supplemented with 10% defibrinated sheep blood and Dents supplement (containing vancomycin, trimethoprim, cefsulodin and amphotericin B) and incubated at 37˚C under microaerophilic conditions using anaerobic jars and strips producing microaerophilic conditions (Campygen strips, Oxoid, UK) for five to seven days. Plates were then examined for bacterial growth and typical colonies were selected for identification.

The identity of *H. pylori* was confirmed by Gram stain, production of urease and catalase. *H. pylori* isolates were defined as Gram-negative spiral-shaped bacilli that were catalase-positive and rapidly (less than 30 min) urease-positive. *H. pylori* ATCC 49503 (type strain) was used as a positive control for the culture conditions and identification tests.

Extraction of genomic DNA

Bacterial cells on a chocolate agar plate were washed twice with phosphate buffer saline (PBS, pH 8.0) then centrifuged at 3000 rpm for 20 min. *H. pylori* DNA was extracted by a phenol/chloroform method similar to a method previously described.[12] Briefly, the bacterial pellet was resuspended in Tris-HCl buffer containing ethylenediaminetetraacetate (TE, pH 8.0) and lysozyme and was then incubated at 37˚C for 30 min. The suspension was treated with sodium dodecyl sulphate (SDS); proteinase K and RNase A. DNA was extracted with phenol/chloroform/isoamyl alcohol, precipitated by sodium acetate and ice-cold absolute alcohol, and washed with ice-cold alcohol (70%). The pellet of DNA was finally resuspended in TE buffer. DNA content

Table 1. Oligonucleotide primers used in typing of *H. pylori*.

Region amplified	Primer designation	Primer sequence (5′ to 3′)	PCR product (bp)	PCR cycles
cagA	D008	GGTCAAAATGCGGTCATGG	297	1 cycle of 94˚C for 5 min, 35 cycles of 94˚C for 1 min, 55°C for 1 min and 72˚C for 90 sec, 1 cycle of 72˚C for 5 min.
	R008	TTAGAATAATCAACAAACATCACGCCAT		
cagAP	cagAP-F1	GTGGGTAAAAATGTGAATCG	730	1 cycle of 94˚C for 5 min followed by 35 cycles of 1 min at 94˚C, 55˚C for 1 min and 72˚C for 1 min. Final cycle of 72˚C for 7 min.
	cagAP-R1	CTGCAAAAGATTGTTTGGCAGA		
***vacA* alleles**				
S1a	SS1-F	GTCAGCATCACACCGCAAC	190	1 cycle of 95˚C for 5 min; 35 cycles of 95˚C for 1 min, 52˚C for 1 min and 72˚C for 1 min; 1 cycle of 72˚C for 5 min.
	VA1-R	CTGCTTGAATGCGCCAAAC		
S1b	SS3-F	AGCGCCATACCGCAAGAG	187	
	VA1-R	CTGCTTGAATGCGCCAAAC		
S2	SS2-F	GCTAACACGCCAAATGATCC	199	
	VA1-R	CTGCTTGAATGCGCCAAAC		
M1	VA3-F	GGTCAAAATGCGGTCATGG	190	
	VA3-R	*CCATTGGTACCTGTAGAAAC3′*		
M2	VA4-F	GGAGCCCCAGGAAACATTG	352	
	VA4-R	CATAACTAGCGCCTTGCAC		
iceA1	iceA1F	GTGTTTTTAACCAAAGTATC	247	1 cycle consisting of 1 min at 95˚C, followed by 35 cycles of 1 min at 95˚C, 1 min at 52˚C, and 1 min at 72˚C. Final cycle of 72˚C for 7 min.
	iceA1R	CTATAGCCASTYTCTTTGCA		
iceA2	iceA2F	GTTGGGTATATCACAATTTAT	229/334	
	iceA2R	TTRCCCTATTTTCTAGTAGGT		

and purity was determined by measuring the absorbance at 260 nm and 280 nm using a spectrophotometer (Beckman DU-600).

Histopathology

Gastric biopsy specimens were stained with haematoxylin and eosin (H&E) to detect *H. pylori* and to assess the degree of gastritis. The presence of *H. pylori* was determined by the positive rapid urease test and histopathology. All biopsy specimens for histological examination were fixed in 10% formalin, processed to paraffin wax and embedded on edge. Sequential sections were cut at 5 m. The degree of acute and chronic inflammation, as well as *H. pylori* density was scored according to the updated Sydney system.[13]

Bacterial density was graded from 0 to 3 (0, absent; 1–3, isolated bacteria to colonies). The infiltration of gastric mucosa by mononuclear cells and polymorphonuclear leucocytes, and presence of atrophy and intestinal metaplasia were graded as follows: 0, none; 1, mild; 2, moderate; 3, marked. Chronic inflammation was defined according to an increase in lymphocytes and plasma cells in the lamina propria, graded into mild, moderate or marked increase in density.

Chronic active gastritis indicated chronic inflammation with neutrophil polymorph infiltration of the lamina propria, pits or surface epithelium and was graded (0, nil; mild, up to a third pits and surface infiltrated; moderate, a third to two-thirds; and marked, two-thirds or above). Antrum and corpus gastritis were scored by total sum of grade of gastritis (mild, 1; moderate, 2; marked, 3 infiltration with lymphocytes and plasma cells) and activity of gastritis (mild, 1; moderate, 2; marked, 3 infiltration with neutrophilic granulocytes) either in the antrum or in the corpus, with a maximum of 6 points for each individual patient.

Atrophy was defined as the loss of inherent glandular tissue, with or without replacement by intestinal-type epithelium. For optimal histological evaluation, all gastric biopsy specimens included surface epithelium and muscularis mucosae. Lymphoid aggregates were defined as accumulations of lymphocytes and plasma cells without a germinal centre.

Table 2. Association of *iceA* type 1 and 2 with clinical outcome.

		iceA type 1			*iceA* type 2		
		Positive (%) *n*=177	Negative (%) *n*=107	*P* value	Positive (%) *n*=158	Negative (%) *n*=126	*P* value
Age (years)							
	18–45	104 (59)	53 (49)	0.130	86 (54)	71 (56)	0.747
	46–79	73 (41)	54 (51)		72 (46)	55 (44)	
Gender							
	Male	131 (74)	86 (80)	0.221	128 (81)	89 (71)	0.041
	Female	46 (26)	21 (20)		30 (19)	37 (29)	
Symptoms							
	Abdominal pain	149 (84)	88 (82)	0.006	125 (79)	112 (89)	0.052
	Haematemesis	14 (8)	1 (1)		9 (6)	6 (5)	
	Melaena	4 (2)	6 (6)		6 (6)	4 (3)	
	Weight loss	10 (6)	12 (11)		18 (11)	4 (3)	
Diagnosis							
	Gastritis	118 (67)	95 (56)		84 (53)	101 (80)	
	Gastric ulcer	29(16)	14 (13)	0.003	34 (21)	9 (7)	<0.001
	Duodenal ulcer	20(11)	6 (5)		12 (8)	14 (11)	
	Gastric carcinoma	10 (6)	20 (19)		28 (18)	2 (2)	
Histopathology							
	Chronic active gastritis	135 (76)	99 (92)	<0.001	139 (88)	95 (75)	0.006
	Chronic gastritis	42 (24)	8 (8)		19 (12)	31 (25)	
Lymphoid aggregates							
	Positive	34 (19)	35 (33)	0.010	42 (27)	27 (21)	0.314
	Negative	143 (81)	72 (67)		116 (73)	99 (79)	
Intestinal metaplasia							
	Positive	12 (7)	2 (2)	0.064	12 (8)	2 (2)	0.020
	Negative	165 (93)	105 (98)		146 (92)	124 (98)	
Inflammation							
	Mild	122 (69)	56 (52)	0.005	90 (57)	88 (70)	0.026
	Moderate	55 (31)	51 (48)		68 (43)	38 (30)	

P<0.05 regarded as significant

Genotyping

Amplification of *cagA*, *cagA*-promoter and *vacA* alleles by PCR was performed in a volume of 25 μL containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol KCl, 1.5–2.5 mmol/L $MgCl_2$, 200 mol/L deoxynucleoside triphosphates, 2 units *Taq* DNA polymerase (Promega) and 25 pmol both forward and reverse primers (Table 1).[14,15] PCR was performed in a Perkin Elmer 9700 thermal cycler. The amplification cycles for *cagA*, *cagA* promoter and *vacA* alleles are given in Table 1.

Positive and negative reagent control reactions were performed with each batch of amplifications. DNA from *H. pylori* strains ATCC 43504 (*vacA s1am1*, *cagA*-positive), ATCC 51932 (*vacA s2m2*, *cagA*-negative) and ATCC 43526 (*vacA s1bm1*, *cagA*-positive) was used to define the accuracy of the *cagA* and *vacA* alleles. After PCR, the amplified products were electrophoresed in 2% agarose gels containing 0.5% Tris/acetate/ethylenediaminetetraacetic acid, stained with ethidium bromide, and visualised under ultraviolet light.

For analysis of the *iceA* genotype, primers previously described by van Doorn *et al*.[6] were used. Primers *iceA*1F and *iceA*1R yielded a fragment of 247 bp for the *iceA*1 allele, and primers *iceA*2F and *iceA*2R yielded a fragment of 229 or 334 bp depending on the presence of repeated sequences of 105 nucleotides.

Statistical analysis

The Statistical Package for Social Science (SPSS, Release 19) was used. The descriptive analysis was performed for demographic and clinical features. Results were presented as mean±standard deviation (SD) for quantitative variables and number (percentage) for qualitative variables. Independent sample Student's *t*-test was used for continuous variables. The χ^2 test or Fisher's exact test was used for categorical variables. Factors found to be statistically significant in univariate analysis were included in a multivariate regression analysis. All *P* values were two sided and significance was set at $P<0.05$.

Results

iceA type 1 was positive in 177 (62%) and *iceA* type 2 in 158 (56%). Single *iceA* type 1 was positive in 115 (40%), type 2 in 96 (34%), both types 1 and 2 were positive in 62 (22%) and both negative in 11 (4%) (Tables 2–4).

Distribution of **iceA** *types in relation to age, gender and symptoms*

H. pylori strains with *iceA* type 2 were more common in male patients: 128 (81%) compared to 89 (71%) for *iceA*-negative ($P=0.041$) (Table 2). Abdominal pain was more common with *iceA* type 1: 149 (84%) compared to 125 (79%) in *iceA* type 2 (Table 2).

Association of **iceA** *types with clinical outcome*

iceA type 1 and type 2 were associated with endoscopic gastritis in 118 (67%) and 84 (53%), respectively (Table 2). In

Table 3. Association of *iceA* types 1 and 2 with *H. pylori* virulence markers.

		iceA type 1			*iceA* type 2		
		Positive (%)	Negative (%)	*P* value	Positive (%)	Negative (%)	*P* value
cagA							
	Positive	105 (59)	53 (49)	0.108	101 (64)	57 (45)	0.002
	Negative	72 (41)	54 (51)		57 (36)	69 (55)	
cagA-promoter							
	Positive	88 (49)	46 (43)	0.271	85 (54)	49 (39)	0.012
	Negative	89 (51)	61 (57)		73 (46)	77 (61)	
vacA							
S1a							
	Positive	119 (67)	75 (70)	0.615	102 (65)	92 (73)	0.128
	Negative	58 (33)	32 (30)		56 (35)	34 (27)	
S1b							
	Positive	26 (15)	10 (9)	0.190	27 (17)	9 (7)	0.012
	Negative	151 (85)	97 (91)		131 (83)	117 (93)	
m1							
	Positive	110 (62)	60 (56)	0.312	99 (63)	71 (56)	0.281
	Negative	67 (38)	47 (44)		59 (37)	55 (44)	
m2							
	Positive	73 (41)	32 (30)	0.055	52 (33)	53 (42)	0.112
	Negative	104 (59)	75 (70)		106 (67)	73 (58)	
S2							
	Positive	61 (35)	47 (44)	0.111	65 (41)	45 (34)	0.227
	Negative	116 (65)	60 (55)		93 (59)	83 (66)	

$P<0.05$ regarded as significant.

iceA type 2, GU was positive in 34 (21%) and GC in 28 (18%) compared to nine (7%) and two (2%) in *iceA* type 2-negative, respectively ($P<0.001$) (Table 2).

Association of iceA *types with histological change*

In *iceA* type 1, chronic active gastritis was present in 135 (76%) compared to 99 (92%) for *iceA* type1-negative ($P<0.001$). *H. pylori iceA* type 2 in 139 (88%) was associated with chronic active gastritis compared to 95 (75%) in *iceA* type 2-negative ($P=0.006$). Comparing *iceA* type 1- and *iceA* type 2-positive cases with negative ones, mild inflammation was more common than moderate inflammation ($P=0.005$ and $P=0.026$, respectively) (Table 2).

Association of iceA *types with* cagA *and* vacA *alleles*

H. pylori iceA type 1 was not associated with *cagA*, *cagA* promoter and *vacA* alleles. *cagA* was positive in 101 (64%) of *iceA* type 2-positive cases compared to 57 (45%) in *iceA* type 2 negative cases ($P=0.002$) (Table 3). The *cagA* promoter was also positive in 85 (54%) of *iceA* type 2-positive cases compared to 49 (39%) in *iceA* type 2-negative cases ($P=0.012$) (Table 3).

H. pylori vacAs1b was positive in 27 (17%) *iceA* type 2-positive cases, compared to nine (7%) in *iceA* type 2-negative cases ($P=0.012$) (Table 3).

Effect of cag positivity on iceA *type 1 and 2 clinical outcome*

In *cagA/iceA* type 1-positive *H. pylori* strains, DU and GU were present in 29 (28%) ($P<0.001$) and 20 (20%) ($P<0.001$), respectively, compared to 14 (8%) and six (3%), respectively, in *cagA/iceA* type 1-negative cases (Table 4). *H. pylori* strains with *cagA/iceA* type 1 and *cagA/iceA* type 2 were associated with GU (odds ratio [OR]: 4.07 [1.9–8.6]; $P<0.001$ and OR: 9.40 [4.2–21.1]; $P<0.001$, respectively) (Table 5). *H. pylori* strains with *cagA/iceA* type 1 were associated with DU (OR: 7.8 [3.0–20.6]; $P<0.001$) (Table 5), while *cagA/iceA* type 2-positives were associated with GC (OR: 18.5 [6.1–56]; $P<0.001$) (Table 5).

Table 4. Association of *cagA-iceA* types 1 and 2 with clinical outcome.

		cagA-iceA type 1			*cagA-iceA* type 2		
		Positive (%) *n*=102	Negative (%) *n*=182	*P* value	Positive (%) *n*=98	Negative (%) *n*=186	*P* value
Age (years)							
	18–45	63 (62)	94 (52)	0.100	56 (57)	101 (54)	0.647
	46–79	39 (38)	88 (48)		42 (43)	85 (46)	
Gender							
	Male	78 (76)	139 (76)	0.985	81 (83)	136 (73)	0.072
	Female	24 (24)	43 (24)		17 (17)	50 (27)	
Symptoms							
	Abdominal pain	74 (72)	163 (90)	<0.001	72 (74)	165 (89)	<0.001
	Haematemesis	14 (14)	1 (1)		8 (8)	7 (4)	
	Melaena	4 (4)	6 (3)		2 (2)	8 (4)	
	Weight loss	10 (10)	12 (6)		16 (16)	6 (3)	
Diagnosis							
	Non-ulcer dyspepsia	43 (42)	142 (78)		30 (31)	155 (83)	
	Gastric ulcer	29 (28)	14 (8)	<0.001	34 (35)	9 (5)	<0.001
	Duodenal ulcer	20 (20)	6 (3)	<0.001	8 (8)	18 (10)	0.071
	Gastric carcinoma	10 (10)	20 (11)	0.234	26 (26)	4 (2)	<0.001
Histopathology							
	Chronic active gastritis	81 (79)	153 (84)	0.334	84 (86)	151 (81)	0.286
	Chronic gastritis	21 (21)	29 (6)		14 (14)	36 (19)	
Lymphoid aggregates							
	Positive	23 (22)	46 (25)	0.607	27 (28)	42 (23)	0.353
	Negative	79 (78)	136 (75)		71 (72)	144 (77)	
Intestinal metaplasia							
	Positive	11 (11)	3 (2)	0.001	12 (12)	2 (1)	<0.001
	Negative	91 (89)	179 (98)		86 (88)	184 (99)	
Inflammation							
	Mild	70 (69)	108 (59)	0.121	54 (55)	124 (67)	0.055
	Moderate	32 (31)	74 (41)		44 (45)	62 (33)	

cagA-iceA positives were positive for both; *cagA-iceA* negatives were either *iceA* or *cagA* negative or negative for both.
$P<0.05$ regarded as significant.

Discussion

This work has shown that in the *H. pylori* strains studied *iceA* type 1 was more common than type 2. There were cases with more than one *iceA* allele type suggesting infection with multiple *H. pylori* strains. This is in keeping with a previous study that showed infection with multiple *H. pylori* strains was frequent in this patient population.[16] *iceA* type 2 was associated with male gender, although this could be due to the greater number of enrolled male patients (Table 2).

Clinical outcome showed gastritis associated with NUD and peptic ulcers were common with *iceA* type 1 while GU and GC were more frequently associated with *iceA* type 2 (Table 2). The mucosal inflammatory changes of chronic active gastritis were predominantly associated with *iceA* type 2 and its severity varied from mild to moderate in *iceA* type 1 and *iceA* type 2 (Table 2). *iceA* type 2 was associated with *cagA* (Table 3). The *cagA/iceA* type 1-positive strains were significantly associated with GU and DU, with 29 (28%) ($P<0.001$) and 20 (20%) ($P<0.001$) compared to 14 (8%) and six (3%), respectively, in *cagA/iceA* type 1-negative cases (Tables 4 and 5). Of the *vacA* alleles only *s1b* was significantly positive in cases with *iceA* type 2 (Table 3). The main shortcoming of this study is the relatively few of cases of *H. pylori*-associated disease (i.e., GU, DU and GC).

The implications of this study are that *iceA* type 1 was predominant among the two *iceA* allele types. *iceA* type 1 was dominant which is in keeping with previous studies from India, the USA and Korea.[5,6,17] *H. pylori* genotypes in gastroduodenal disease in a population from Western India demonstrated that 71% were *cagA*-positive, the *vacA s1* and *m1* alleles were found in 54% and 59% patients, respectively, while *iceA* type 1 was present in 40% and *iceA* type 2 in 13% patients.[17] *iceA* type 2 was present in 56% of our *H. pylori* isolates, which is higher than the 13.5% and 15% reported in regional studies from India and Malaysia, respectively.[17,18]

Table 5. Logistic regression analysis for *iceA* allele with virulence marker and clinical findings.

	Odds ratio (95% CI)	*P* value
Gastric ulcer		
cagA-iceA type 1		
Negative	1	
Positive	4.07 (1.92–8.60)	<0.001
cagA-iceA type 2		
Negative	1	
Positive	9.40 (4.19–21.1)	<0.001
Duodenal ulcer		
cagA-iceA type 1		
Negative	1	
Positive	7.8 (3.0–20.6)	<0.001
Gastric carcinoma		
cagA-iceA type 2		
Negative	1	
Positive	18.5 (6.1–56.0)	<0.001

$P<0.05$ regarded as significant.
cagA-iceA positives were positive for both.

Mild gastritis was more commonly associated with *iceA* type 1 while chronic active gastritis and chronic gastritis of moderate severity were associated with *iceA* type 2. Those with *iceA* type 2 may also be *cagA*-, *cagA* promoter- and *vacA s1b*-positive, which were associated with GU and GC. Gastric ulcer was more common with *iceA* type 2 while DU was associated with *cagA/iceA* type 1-positive case.

In this study, *cagA* was positive in 64% cases with *iceA* type 2 (Table 4). We found *iceA* type 1 association with peptic ulcer disease, described in previous studies with *cagA*-positive isolates. This is different to a previous study which identified *cagA* as an independent marker of peptic ulcer disease.[6] *iceA* type 2 was predominant in GC, which is comparable to the findings of a study from Colombia.[6] In a meta-analysis that examined 15 studies for the association between the presence of *cagA* and *iceA* allele types, only two showed a positive correlation of *cagA* with *iceA* type 1 and *iceA* type 2.[19,20]

This is the first report of *iceA* allele types in a local Pakistani population. Overall, our results demonstrated *H. pylori* isolate genetic affinities with Western and Asian gene pools, with some distinctive genetic features of virulence genes that may have evolved in the local population. *H. pylori* isolates with the genotype *cagA/iceA* type 1 offered an increased chance of developing GU or DU, while *cagA/iceA* type 2-positives were associated with GU and GC.

In conclusion, we were able to show that *iceA* allele types were significantly associated with *H. pylori*-related peptic ulcer disease and GC. *H. pylori* infection with *cagA/iceA* type 2 signified serious *H. pylori*-related disease in the patient group. □

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