Original Article

Comparison of the Virulence Markers of *Helicobacter Pylori* and their Associated Diseases in Patients from Pakistan and Afghanistan

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ABSTRACT

Background/Aim: *Helicobacter pylori* is a Gram-negative bacteria, which is associated with development of gastroduodenal diseases. The prevalence of *H. pylori* and the virulence markers cytotoxin-associated gene A and E (*cagA*, *cagE*) and vacuolating-associated cytotoxin gene (*vacA*) alleles varies in different parts of the world. *H. pylori* virulence markers *cagA, cagE*, and *vacA* alleles in local and Afghan nationals with *H. pylori*-associated gastroduodenal diseases were studied. **Patients and Methods:** Two hundred and ten patients with upper gastrointestinal symptoms and positive for *H. pylori* by the urease test and histology were included. One hundred and nineteen were local nationals and 91 were Afghans. The *cagA*, *cagE*, and *vacA* allelic status was determined by polymerase chain reaction. **Results:** The nonulcer dyspepsia (NUD) was common in the Afghan patients ($P = 0.025$). In Afghan *H. pylori* strains, *cagA* was positive in 14 (82%) with gastric carcinoma (GC) compared with 29 (45%) with NUD ($P = 0.006$), whereas *cagE* was positive in 11 (65%) with GC and 4 (67%) with duodenal ulcer (DU) compared with 12 (18%) with NUD ($P < 0.001$ and 0.021, respectively). The *vacA s1a/b1*was positive in 10 (59%) of GC compared with 20 (31%) in NUD ($P = 0.033$). In Pakistani strains, *cagE* was positive in 12 (60%) with GC, 7 (58%) with GU, 12 (60%) with DU compared with 11 (16%) with NUD ($P < 0.001$, 0.004, and < 0.001, respectively). In Pakistani strains, *cagA/s1a/m1* was 39 (33%) compared with Afghans in 17 (19%) ($P = 0.022$). Moderate to severe mucosal inflammation was present in 51 (43%) Pakistani patients compared with 26 (28%) ($P = 0.033$) in Afghans. It was also associated with grade 1 lymphoid aggregate development in Pakistani patients 67 (56%) compared with 36 (40%) ($P = 0.016$) in Afghans. **Conclusion:** Distribution of *H. pylori* virulence marker *cagE* with DU was similar in Afghan and Pakistan *H. pylori* strains. Chronic active inflammation was significantly associated with Pakistani *H. pylori* strains.

Key Words: *cagA*, *cagE*, gastritis, gastric carcinoma, *Helicobacter pylori*

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Helicobacter pylori is a Gram-negative bacteria that inhabit the gastric mucosal lining. Adhesion of the bacteria to the gastric mucosa is a necessary prerequisite for the pathogenesis of H. pylori-related diseases. Although most patients are asymptomatic, persistent infection may cause chronic gastritis, gastric ulcer, gastric cancer, and duodenal ulcer. The prevalence varies among countries with existing evidence suggesting that the diversity in disease outcome may be ascribed to variations in infecting strains.[1,2] The virulence markers of *H. pylori*, such as cytotoxin-associated genes A (*cag*A) and E (*cag*E), *vacuolating cytotoxin* (*vac*A) and its alleles have been shown to be associated with its various manifestations.[3] *H. pylori* genotypes and their geographic distribution are linked to the severity of peptic ulcer disease (PUD).[4,5] The *H. pylori* genome is genetically diverse, as it can be seen in the cag pathogenicity island (PAI) and allelic variation within the *vac*A gene.[5,6] The cytotoxin-associated gene A (*cag*A) has been proposed as a marker for the cag PAI and is associated with more severe clinical outcomes.[3-5] The cag PAI genes contain a *cagE* gene that encodes a secretory protein that is required for the induction of interleukin-8 and for translocation and phosphorylation of CagA protein.[7,8] The *cagE* genotype has been associated with gastric cancer in

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Table 1: Clinical details, histological changes, and *Helicobacter pylori* virulence markers in the groups

	Pakistan *n*=119	Afghanistan *n*=91	*P* value
Age (years)			
Mean±SD	45±16	43±14	
Range	18-83	19-75	
Sex			
Male	69 (58)	65 (71)	0.045*
Female	50 (42)	26 (29)	
Symptoms			
Abdominal pain	103 (86)	82 (90)	0.704
Reflux	2 (2)	2 (2)	
Nausea	6 (5)	4 (4)	
Hematemesis	8 (7)	3 (3)	
Diagnosis			
Nonulcer dyspepsia	67 (56)	65 (71)	0.025*
Gastric ulcer	12 (10)	3 (3)	
Duodenal ulcer	20 (17)	6 (7)	
Gastric carcinoma	20 (17)	17 (19)	
Virulence markers			
cagA			
Positive	62 (52)	47 (52)	0.948
Negative	57 (48)	44 (48)	
cagE			
Positive	42 (35)	29 (32)	0.603
Negative	77 (65)	62 (68)	
vacAs1a			
Positive	79 (66)	55 (60)	0.374
Negative	40 (34)	36 (40)	
vacAs1b			
Positive	40 (34)	32 (35)	0.814
Negative	79 (66)	59 (65)	
vacAm1			
Positive	78 (66)	48 (53)	0.061
Negative	41 (34)	43 (47)	
vacAm2			
Positive	54 (45)	46 (51)	0.457
Negative	65 (55)	45 (49)	
s1a/m1			
Positive	55 (46)	38 (42)	0.156
Negative	64 (54)	53 (58)	
s1b/m1			
Positive	26 (22)	18 (20)	0.660
Negative	93 (78)	73 (80)	
s1a/m2			
Positive	35 (29)	24 (26)	0.321
Negative	84 (71)	67 (74)	
s1b/m2			
Positive	19 (16)	16 (18)	0.715
Negative	100 (84)	75 (82)	
cagA/s1a/m1			
Positive	39 (33)	17 (19)	0.022[a]
Negative	80 (67)	74 (78)	
cagA/s1b/m1			
Positive	16 (13)	14 (15)	0.691
Negative	103 (87)	77 (85)	
cagA/s1a/m2			
Positive	16 (13)	7 (8)	0.186
Negative	103 (87)	84 (92)	
cagA/s1b/m2			
Positive	5 (4)	7 (8)	0.280
Negative	114 (96)	84 (92)	

Results are presented as mean±standard deviation for quantitative variables and number (percentage) for qualitative variables. Differences in proportion were assessed by using Pearson Chi-square test, Fisher exact test, or likelihood ratio test where appropriate, [a]*P* value less than 0.05 was considered as statistically significant

some studies but contrary results have also been published.[9] *Vacuolating cytotoxin* A (*vac*A) is present in all *H. pylori* bacteria and has two variable parts, the signal or s-region, and the middle or m-region.[10] The "s" region and "m" region can be differentiated into *s1a*, *s1b*, *s1c*, *s2* and *m1a*, *m1b*, *m1c*, and *m2* subtypes, respectively. The different combination of s- and m-region allelic types determines the structure of the cytotoxin. Moreover, there is variability in *vac*A in the intermediate (i)-region.[11] The *vac*A "*s1*" and "*m1*" strains are associated with greater gastric epithelial damage than "*s2*" and "*m2*" strains.[10] *Vac*A *s1a/m1* strains are more pathogenic than *s2/m2* strains.[11]

The prevalence of *H. pylori* is high in developing countries. Its seroprevalence in Pakistan exceeds 58% of general population and is common in asymptomatic populations.[12] Pakistan and Afghanistan are neighboring countries and many Afghan citizens avail health-care facilities within pakistan. Studies about the seroprevalence of *H. pylori* in Afghanistan population are lacking but it appears to be common in view of high incidence of infections having feco-oral route of transmission. Poor quality of water supply and breakdown of infrastructure, including sanitary conditions, may contribute to high prevalence of this bacterium. Although there are several recent studies examining the relationship between *H. pylori* virulence factors and clinical outcomes in Pakistan,[13,14] there is no study that has compared the virulence marker of Pakistani and Afghan *H. pylori* strains. The distribution of *cag*A, *cagE*, and *vac*A alleles in Pakistani and Afghan *H. pylori* strains from patients with upper gastrointestinal symptoms were compared and their association with clinical diagnosis was studied.

PATIENTS AND METHODS

Patients

Two hundred and ten patients were included in the

study. All the patients were reported positive for *H. pylori* infection by the rapid urease test and histology. They included 119 patients who were local nationals (69 males and 50 females with a mean age of 45 years) and 91 Afghan patients who recently travelled to Pakistan to seek health care (65 males and 26 females with a mean age of 43 years) [Table 1]. They attended the gastroenterology outpatient and endoscopy suite from June 2008 to June 2011. All presented with upper gastrointestinal symptoms and they were diagnosed as having nonulcer dyspepsia (NUD), gastric ulcer (GU), gastric carcinoma (GC), and duodenal ulcer (DU) [Table 1]. The GC were distributed in body in 22 patients (11%), in antrum 12 (6%), and in fundus in 3 patients (1%), respectively. They were adenocarcinomas: 24 were diffuse and 13 intestinal. The study was approved by the institutional ethics review committee. All patients gave an informed consent for endoscopy and participation in the study. None of the patients had received previous treatment for *H. pylori* infection, antibiotics, acid-reducing drugs, such as H2-receptor antagonists, acid pump inhibitors, nonsteroidal anti-inflammatory drugs, or bismuth compounds in the last four weeks. The clinical symptoms at the time of presentation and endoscopic findings were noted. Gastric biopsy specimens were taken from an area of inflammation in the antrum and corpus. Two biopsy specimens were removed for each of the rapid urease test, histology, and polymerase chain reaction (PCR). Specimens for histology were dispatched in formalin, whereas for PCR in 0.9% normal saline. The *cag*A PCR for 5′ terminal, *cagE* and *vac*A alleles for the signal "*s*" and middle "*m*" were analyzed.

Urease test

The tissue specimens were used for the rapid urease test (Pronto Dry, Brignais, France) results were read in 30 min after sampling as directed by the manufacturer. The color change from yellow to pink was considered positive.[15]

Histology

Gastric biopsy specimens for histopathology were stained using hematoxylin and eosin (stain for the detection of *H. pylori*), and the degree of gastritis was scored in accordance with the Sydney system.[16] The bacterial density was graded from 0 to 3 (0, absent; 1-3, from few and isolated bacteria to colonies). The infiltration of gastric mucosa by mononuclear cells and polymorphonuclear leukocytes, atrophy, and intestinal metaplasia (IM) 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 neutrophilic polymorph infiltration of the lamina propria, pits, or surface epithelium graded as 0 = nil, mild ≤1/3 of pits and surface infiltrated; moderate = 1/3 to 2/3; and marked ≥2/3. Atrophy was defined as the loss of inherent glandular tissue, with or without replacement by intestinal-type epithelium. Lymphoid aggregates were defined as accumulations of lymphocytes and plasma cells without a germinal center.

DNA extraction from tissues

DNA was extracted from gastric tissue as described previously.[17] Briefly, gastric tissue was homogenized in sterile water and centrifuged. Lysis buffer (100 mM NaCl, 10 mM Tris–HCl [pH 8.0], 25 mM ethylenediaminetetraacetic acid (EDTA), 0.5% sodium dodecyl sulfate) and 10 μL of Proteinase K (10 mg/mL) was added followed by incubation at 50°C for 20 h. DNA was extracted by phenol–chloroform extraction and ethanol precipitation. The resulting pellet was dissolved in 40 μL of Tris–HCl and EDTA containing buffer (10 mM Tris–HCl [pH 7.4] and 0.1 mM EDTA [pH 8.0]). Samples were stored at −20°C before PCR amplification. DNA content and purity was determined by measuring the absorbance at 260 and 280 nm using a spectrophotometer (Beckman DU-600, Michigan, USA).

Table 2: Oligonucleotide primers used in typing of *Helicobacter pylori cagA*, *cagE*, and *vacA* alleles

Region amplified	Primer designation	Primer sequence (5′-3′)	Size of PCR product	PCR cycles
CagA	D008	GGTCAAAATGCGGTCATGG	297-bp[18]	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 s, 1 cycle of 72°C for 5 min
	R008	TTAGAATAATCAACAAACATCACGCCAT		
CagE	F1	5′-GCGATTGTTATTGTGCTTGTAG-3′	329-bp[19]	
	R1	5′-GAAGTGGTTAAAAAATCAATGCCCC-3′		
Vac A alleles				
S1a	SS1-F	GTCAGCATCACACCGCAAC	190-bp[18]	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-bp[18]	
	VA1-R	CTGCTTGAATGCGCCAAAC-		
m1	VA3-F	GGTCAAAATGCGGTCATGG	290-bp[18]	
	VA3-R	CCATTGGTACCTGTAGAAAC3′		
m2	VA4-F	GGAGCCCCAGGAAACATTG	352-bp[18]	
	VA4-R	CATAACTAGCGCCTTGCAC		

Polymerase chain reaction

Amplification of *cag*A, *cagE*, and *vac*A alleles by PCR was performed in a volume of 50 μL containing 10 mM/L Tris–HCl (pH 8.3), 50 mM/L KCl, 1.5-2.5 mM/L $MgCl_2$, 200 mM/L deoxynucleoside triphosphates, 2 units *Taq* DNA polymerase (Promega, Wisconsin, USA) and 25 pmol of both forward and reverse primers [Table 2] used before[18,19] (synthesized by MWG Automatic synthesizer, Huntsville, USA). PCR was performed in a Perkin Elmer 9700 thermal cycler. The amplification cycles for *cag*A and *vac*A alleles are given in Table 2. Positive and negative reagent control reactions were performed with each batch of amplifications. DNA from *H. pylori* strains ATCC 43504 (*vac*A*s1a/m1*, *cag*A positive), ATCC 51932 (*vac*A *s2/m2*, *cag*A negative), and ATCC 43526 (*vac*A *s1b/m1*, *cag*A positive) was used to define the accuracy of the *cag*A. After PCR, the amplified PCR products were electrophoresed in 2% agarose gels containing 0.5% × Tris/acetate/EDTA, stained with ethidium bromide, and visualized under a short-wavelength ultraviolet light source.

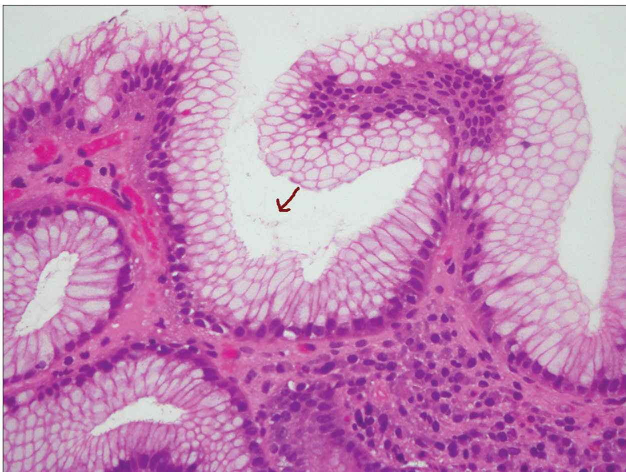

Figure 1: Gastric mucosa showing a number of *Helicobacter pylori* organisms (H and E, ×20)

Statistical assessment

The statistical package for social science SPSS (Release 16, standard version, copyright © SPSS; 2007) was used for data analysis. The descriptive analysis was done for demographic and clinical features. Results were presented as mean ± standard deviation for quantitative variables and number (percentage) for qualitative variables. Differences in proportion were assessed by using Pearson Chi-square test, Fisher exact test, or Likelihood ratio test where appropriate. *P* value less than 0.05 was considered as statistically significant.

RESULTS

The mean age and range of the Pakistani and Afghan patients were similar. There was a significant difference in the gender of Afghan patients as there were more males

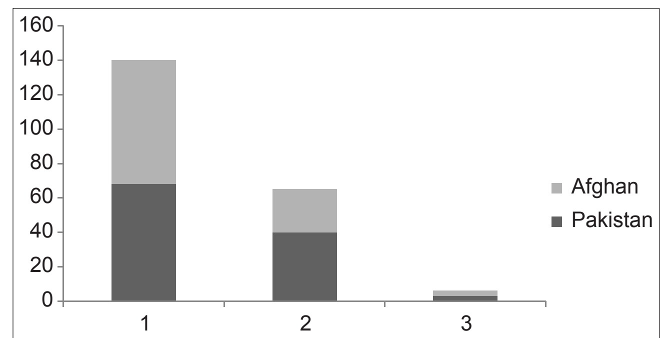

Figure 2: Inflammatory activity associated with *Helicobacter pylori* from grades 1-3 in Pakistan and Afghan patients (*P* = 0.033)

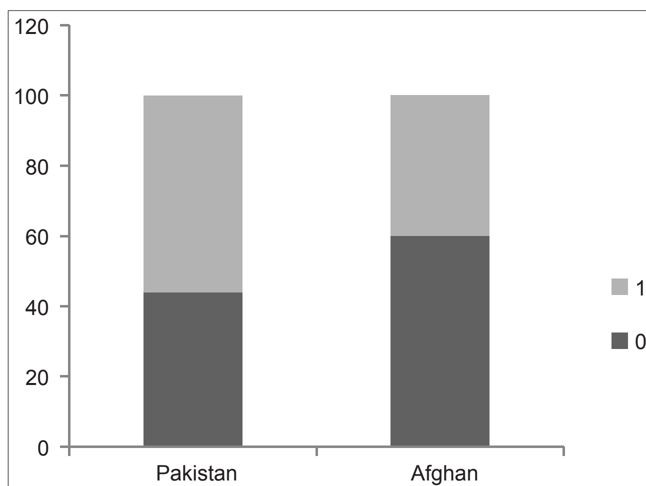

Figure 3: Gastric mucosal lymphoid aggregate formation associated with *Helicobacter pylori* varying from grades 0-1 in Pakistan and Afghan patients (*P* = 0.016)

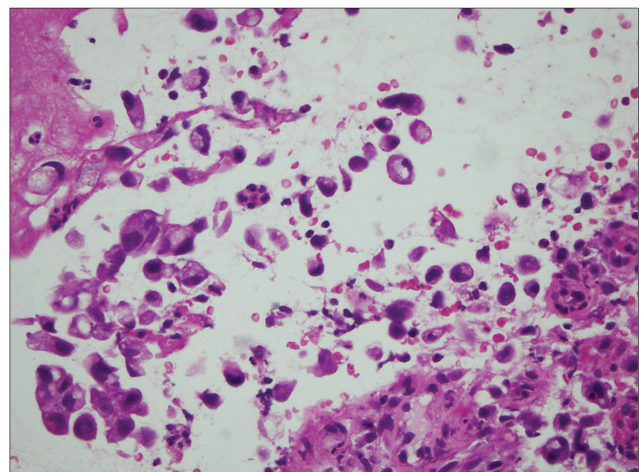

Figure 4: Signet ring carcinoma of the gastric epithelium showing peripheral nuclei and empty cytoplasm (H and E, ×20)

65 out of 91 (71%) compared with Pakistani patients ($P = 0.045$) [Table 1]. There was no significant difference in the distribution of symptoms in the two groups ($P = 0.704$) [Table 1]. The endoscopic diagnosis of NUD was significantly more common in the Afghan patients compared with Pakistanis ($P = 0.025$); 27% (32/119) Pakistani patients had PUD compared with 10% (9/91) Afghan patients ($P = 0.002$), whereas GC was diagnosed at a similar frequency in the two groups [Table 1].

Comparison of *H. pylori* genotypes in groups

The distribution of *cag*A and *cagE* was similar in Afghan and Pakistani *H. pylori* strains [Table 1]. In Pakistani *H. pylori* strains, *vacAm1* was positive in 78 (66%) compared with 48 (53%) in the strains in Afghans, whereas *cagA/s1a/m1* was more frequently found in Pakistani *H. pylori* strains 39 (33%) compared with 17 (19%) ($P = 0.025$) in Afghan strains.

Comparison of histological changes in groups

The density of *H. pylori* and neutrophil infiltration on histology was similar in the two groups [Figure 1]. Grade 1 inflammation was present in 65 (72%) Afghan patients compared with 68 (57%) ($P = 0.033$) in Pakistani patients [Figure 2]. It was also associated with Grade 1 lymphoid aggregate in Pakistani patients 67 (56%) compared with 36 (40%) ($P = 0.016$) in Afghans [Figure 3].

Correlation of *H. pylori* genotypes with diagnosis

In Afghan *H. pylori* strains, *cag*A was positive in 14 (82%) with GC compared with 29 (45%) with NUD ($P = 0.006$), whereas *cagE* was positive in 11 (65%) with GC and 4 (67%) with DU compared with 12 (18%) with NUD ($P < 0.001$ and 0.021, respectively). The *vac*A *s1a/b1* allele was present in 10 (59%) of GC compared with 20 (31%) in NUD ($P = 0.033$). The *H. pylori* genotype cag*A/vac*A*s1a/m1*

Table 3: Distribution of *Helicobacter pylori* virulence markers in associated disease groups

Virulence marker	Pakistan *n*=119 (57)					Afghanistan *n*=91 (43)				
	Nonulcer dyspepsia *n*=67	Gastric ulcer *n*=12	Gastric carcinoma *n*=20	Duodenal ulcer *n*=20	*P* value	Nonulcer dyspepsia *n*=65	Gastric ulcer *n*=3	Gastric carcinoma *n*=17	Duodenal ulcer *n*=6	*P* value
CagA										
Positive	38 (57)	7 (58)	16 (80)	12 (60)	0.274	29 (45)	2 (67)	14 (82)	2 (83)	0.024*
Negative	29 (43)	5 (42)	4 (20)	8 (40)		36 (55)	1 (33)	3 (18)	4 (17)	
CagE										
Positive	11 (16)	7 (58)	12 (60)	12 (60)	<0.001	12 (18)	2 (67)	11 (65)	4 (67)	<0.001*
Negative	56 (84)	5 (42)	8 (40)	8 (40)		53 (82)	1 (33)	6 (35)	2 (33)	
VacAs1a										
Positive	38 (57)	9 (75)	18 (90)	14 (70)	0.025	37 (57)	3 (100)	11 (65)	4 (67)	0.31*
Negative	29 (43)	3 (25)	2 (10)	6 (30)		28 (43)	0 (0)	6 (35)	2 (33)	
VacAs1b										
Positive	23 (34)	5 (42)	5 (25)	7 (35)	0.785	20 (31)	1 (33)	10 (59)	1 (17)	0.13*
Negative	44 (66)	7 (58)	15 (75)	13 (65)		45 (69)	2 (67)	7 (41)	5 (83)	
VacAm1										
Positive	35 (52)	10 (83)	18 (90)	15 (75)	0.003	29 (45)	2 (67)	12 (71)	5 (83)	0.08*
Negative	32 (48)	2 (17)	2 (10)	5 (25)		36 (55)	1 (33)	5 (29)	1 (17)	
VacAm2										
Positive	33 (49)	6 (50)	6 (30)	9 (45)	0.490	35 (54)	1 (33)	7 (41)	3 (50)	0.74*
Negative	34 (51)	6 (50)	14 (70)	11 (55)		30 (46)	2 (67)	10 (59)	3 (50)	
Vacs1/am1										
Positive	21 (31)	8 (67)	14 (70)	12 (60)	0.003#,	24 (37)	2 (67)	8 (47)	4 (67)	0.38*
Negative	46 (69)	4 (33)	6 (30)	8 (40)		41 (63)	1 (33)	9 (53)	2 (33)	
Vacs1b/m1										
Positive	14 (21)	4 (33)	3 (15)	5 (25)	0.663	9 (14)	1 (33)	7 (41)	1 (17)	0.113*
Negative	53 (79)	8 (67)	17 (85)	15 (75)		56 (86)	2 (67)	10 (59)	5 (83)	
CagA/vacs1a/m1										
Positive	16 (24)	3 (25)	13 (65)	7 (35)	0.009	9 (14)	2 (67)	8 (47)	1 (17)	0.013*
Negative	51 (76)	9 (75)	7 (35)	13 (65)		56 (86)	1 (33)	9 (53)	5 (83)	
CagA/vacs1bm1										
Positive	6 (9)	2 (17)	3 (15)	5 (25)	0.342	7 (11)	0 (0)	7 (41)	1 (17)	0.035*
Negative	61 (91)	10 (83)	17 (85)	15 (75)		58 (89)	3 (100)	10 (59)	5 (83)	

Differences in proportion were assessed by using Pearson Chi-square test# and Likelihood-ratio test*, *P* value less than 0.05 was considered as statistically significant

Table 4a: Correlation of histological changes with *Helicobacter pylori cagA* and *cagE* genotypes in different groups

Histology	Pakistan *n*=119 (57)						Afghanistan *n*=91 (43)					
	CagA			*CagE*			*CagA*			*CagE*		
	Positive	Negative	*P* value	Positive	Negative	*P* value	Positive	Negative	*P* value	Positive	Negative	*P* value
Inflammation												
1	41 (56)	27 (59)	0.954*	22 (52)	46 (60)	0.454*	35 (75)	30 (68)	0.063*	21 (72)	44 (71)	0.388*
2	30 (41)	18 (39)		18 (43)	30 (39)		9 (19)	14 (32)		6 (21)	17 (27)	
3	2 (3)	1 (2)		2 (5)	1 (1)		3 (6)	0 (0)		2 (7)	1 (2)	
Neutrophil infiltration												
1	19 (26)	18 (39)	0.140*	7 (16)	30 (39)	0.006*	9 (19)	16 (36)	0.033*	4 (14)	21 (34)	0.067*
2	52 (71)	28 (61)		33 (79)	47 (61)		35 (75)	28 (64)		23 (79)	40 (64)	
3	2 (3)	0 (0)		2 (5)	0 (0)		3 (6)	0 (0)		2 (7)	1 (2)	
Lymphocyte aggregate												
0	25 (34)	27 (59)	0.009#	14 (33)	38 (49)	0.122#	29 (62)	26 (59)	0.799#	19 (65)	36 (26)	0.498#
1	48 (66)	19 (41)		28 (67)	39 (51)		18 (38)	18 (41)		10 (35)	26 (42)	

Differences in proportion were assessed by using Pearson Chi-square test# or Likelihood ratio test* where appropriate. *P* value less than 0.05 was considered as statistically significant

was associated in 8 (47%) with GC compared with 9 (14%) in NUD (*P* = 0.006) [Table 3]. The *H. pylori* genotype *cagA/vacAs1b/m1* was associated with GC in 7 (41%) compared with 7 (11%) in NUD (*P* = 0.007) [Figure 4 and Table 3].

In Pakistani *H. pylori* strains, *cagA* did not achieve significant distribution as the number of patients were less in each of the three diagnosis of GU and GC and also DU (*P* = 0.274) [Table 3]. *cagE* was positive in 12 (60%) with GC, 7 (58%) with GU, 12 (60%) with DU compared with 11 (16%) with NUD (*P* < 0.001, 0.004, and < 0.001 respectively) [Table 3]. The *vacA* allele "*s1a*" was positive in 18 (90%) with GC compared with 38 (57%) in NUD (*P* = 0.006), whereas "*m1*" was positive in patients with DU in 18 (90%) and 10 (83%) in GU compared with 35 (52%) in NUD (*P* = 0.002 and 0.045, respectively). The *H. pylori vacAs1a/m1* was associated with GC in 14 (70%), 12 (60%) in DU, and 8 (67%) in GU compared with 21 (31%) in NUD (*P* = 0.002, 0.020, and 0.026, respectively). The *H. pylori* genotype *cagA/vacAs1a/m1* was associated in 13 (65%) with GC compared with 16 (24%) in NUD (*P* = 0.001).

Correlation of histological changes with *H. pylori* genotypes

Marked gastritis was associated with *cagA* among Afghans compared with Pakistani patients [Table 4a]. *CagE* was associated with neutrophil infiltration in both the groups [Table 4a]. Lymphocyte aggregation was significantly associated with *cagA* in Pakistanis compared with Afghan patients. The distribution of *vacA* alleles was not different among Afghan and Pakistani *H. pylori* strains except that *vacAs1b/m1* and *cagA/s1b/m1* were significantly distributed among Afghan *H. pylori* strains compared with Pakistani *H. pylori* strains, *P* = 0.010 and 0.001, respectively [Table 4b]. There were two cases of chronic atrophic gastritis among Afghan patients and five among Pakistani patients, and they were equally distributed at the antrum and corpus of the stomach in the two groups. There were four cases of IM documented in Pakistani patients, whereas none was documented in Afghan patients.

DISCUSSION

This study showed that both Afghan and Pakistani *H. pylori* strains were associated with NUD in majority of the patients. The density of *H. pylori* strains did not vary in the two groups but Pakistani *H. pylori* strains exceeded in their association with moderately active inflammation and lymphocyte aggregate formation compared with Afghan strains. The distribution of virulence markers *cagA*, *cagE*, and *vacA* alleles were similar in the Afghan and Pakistan H. pylori strains and *H. pylori cagE* was associated strongly with GC and DU compared with NUD, respectively. There was a difference in the *vacA* signal "s" and middle "m" region types between Afghan and Pakistani *H. pylori* strains. Among the Afghan strains, *vacA* genotypes *s1a* and *m1* did not show association with peptic ulcer and GC compared with Pakistani strain. However, *vacAs1b/m1* allele in Afghan *H. pylori* strains was associated with GC. In comparison, *vacA* alleles "*s1a*" and "*m1*" were significantly associated with GC and peptic ulcer, respectively, compared with NUD in Pakistan strains. *H. pylori cag/s1a/m1* was significantly associated with GC in Pakistani and GU in Afghan *H. pylori* strains.

In an earlier study, genotypes of *H. pylori* isolates obtained from 15 Afghan immigrants in Iran, the *cagA* was positive in 60% and *cagE* in 53% of Afghan isolates, while the most common *vacA s*-region genotype was *s1* in 80% and the *s1/m1* was observed in 53%.[20] However, there was no significant association found between *cagA*, *cagE*, and *vacA* genotypes and clinical outcomes in Iranian and

Table 4b: Correlation of histological changes with *Helicobacter pylori vacA* alleles in different groups

Histology	*VacAs1a/m1*			*VacAs1b/m1*			*CagA/s1a/m1*			*CagA/s1b/m1*		
	Positive	Negative	*P* value	Positive	Negative	*P* value	Positive	Negative	*P* value	Positive	Negative	*P* value
Pakistan (*n*=119)												
Inflammation												
1	30 (55)	38 (59)	0.710*	17 (65)	51 (55)	0.343*	18 (46)	50 (63)	0.231*	12 (75)	56 (54)*	0.218
2	23 (42)	25 (39)		9 (35)	39 (42)		20 (51)	28 (35)		4 (25)	44 (43)	
3	2 (4)	1 (2)		0 (0)	3 (3)		1 (3)	2 (2)		0 (0)	3 (3)	
Neutrophil infiltration												
1	20 (36)	17 (27)	0.504*	6 (23)	31 (33)	0.337*	10 (26)	27 (34)	0.605*	3 (19)	34 (33)	0.346*
2	34 (62)	46 (72)		20 (77)	60 (65)		28 (72)	52 (65)		13 (81)	67 (65)	
3	1 (2)	1 (2)		0 (0)	2 (2)		1 (2)	1 (1)		0 (0)	2 (2)	
Lymphocyte aggregate												
0	21 (38)	31 (48)	0.261#	12 (46)	40 (43)	0.775#	14 (36)	38 (47)	0.231#	4 (25)	48 (47)	0.105#
1	34 (62)	33 (52)		14 (54)	53 (57)		25 (64)	42 (53)		12 (75)	55 (53)	
Afghanistan (*n*=91)												
Inflammation												
1	25 (66)	40 (75)	0.498*	11 (61)	54 (74)	0.173*	12 (60)	53 (75)	0.179*	10 (67)	55 (73)	0.134*
2	11 (29)	12 (23)		5 (28)	18 (25)		6 (30)	17 (24)		3 (20)	20 (26)	
3	2 (5)	1 (2)		2 (11)	1 (1)		2 (10)	1 (1)		2 (13)	1 (1)	
Neutrophil infiltration												
1	13 (34)	12 (23)	0.281*	1 (6)	24 (33)	0.010*	5 (25)	20 (28)	0.239*	0 (0)	25 (33)	0.001*
2	23 (61)	40 (75)		15 (83)	48 (66)		13 (65)	50 (71)		13 (87)	50 (66)	
3	2 (5)	1 (2)		2 (11)	1 (2)		2 (10)	1 (2)		2 (13)	1 (1)	
Lymphocyte aggregate												
0	27 (71)	28 (53)	0.080#	8 (44)	47 (64)	0.121#	14 (70)	41 (58)	0.322#	7 (47)	48 (63)#	0.233
1	11 (29)	25 (47)		10 (56)	26 (36)		6 (30)	30 (42)		8 (53)	28 (37)	

Differences in proportion were assessed by using *Likelihood ratio test or Pearson Chi-square test#. *P* value less than 0.05 was considered as statistically significant

Afghan patients.[20] In both Afghan and Pakistani strains, all *cagE*-positive strains also typed positive for *cagA*. The limitation of this study is that small number of strains from patients with peptic ulcer and gastric cancer was evaluated, which is rather small to reveal differences. However, the study shows that Afghan *H. pylori* strains are not more virulent than Pakistani strains as *cagA*- and *cagE*-positive strains were equally common in both the groups (52% vs. 52% and 35% vs. 32%, respectively). There was a strong association between *H. pylori* virulence marker and disease in Pakistani but not in Afghan patients suggesting that other host and environmental factors may be more important in the disease process in Afghan patients.

The study of *H. pylori* virulence factors in populations is important, as they contribute to disease risk. According to the latest World Health Organization data published in April 2011, stomach cancer deaths in Pakistan reached 6541 or 0.51% of total deaths with the age-adjusted death rate of 6.66 per 100,000 of population, ranking Pakistan number 97 in the world.[21] In comparison, stomach cancer deaths in Afghanistan reached 1604 or 0.44% of total deaths with the age-adjusted death rate of 17.07 per 100,000 of population ranking Afghanistan at number 20 in the world.[22] The gastric cancer rate in Pakistan is high compared with that in Afghanistan. In the absence of an East Asian- (eg., China) type universally virulent strains, this gastric cancer rate in Pakistan appears to be lower than that of stomach cancer deaths in China of 3.99% and Iran 2.34%, respectively, of total deaths.[23,24] In conclusion, distribution of *H. pylori* virulence marker *cagE* with DU was similar in Pakistan and Afghan *H. pylori* strains. Chronic active inflammation was significant in association with Pakistani *H. pylori* strains.

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