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 (cagA) and E (cagE), vacuolating cytotoxin (vacA) 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 vacA gene.\(^6,7\) The cytotoxin-associated gene A (cagA) 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

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

|                           | Pakistan | Afghanistan | P value |
|---------------------------|----------|-------------|---------|
| Age (years)               |          |             |         |
| Mean±SD                   | 45±14    | 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*  |
| Negative                  | 80 (67)  | 74 (78)     |         |

Table 1: Contd...

|                           | Pakistan | Afghanistan | P value |
|---------------------------|----------|-------------|---------|
| cagA/s1b/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. *P* value less than 0.05 was considered as statistically significant.

There is no study that has compared some studies but contrary results have also been published.[9] Vacuolating cytotoxin A (*vacA*) 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 *vacA* in the intermediate (i)-region.[11] The *vacA* “s1” and “m1” strains are associated with greater gastric epithelial damage than “s2” and “m2” strains.[10] *VacA 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 *cagA*, *cagE*, and *vacA* 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 *cagA* PCR for 5’ terminal, *cagE* and *vacA* 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.

**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. 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. 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               | GGTCAGAAATGGCGTATGG     | 297-bp*             | 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               | TTAGAATATACCAAAATCATCACGCAT |                    |            |
| CagE             | F1                 | 5’-GCCATTGTATTTGCTTGTAG-3’ | 329-bp*             | 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 |
|                  | R1                 | 5’-GAAGTGGTTTTGCTTGTAG-3’ | 352-bp               |            |
| Vac A alleles    | S1a                | GTCAAGCATCACACCGGCAAC    | 190-bp*             | 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              | CGTCTTGAATGGCGCAAC      | 187-bp*             |            |
|                  | S1b                | AGGCCCATACCGCGAAG       | 290-bp*             |            |
|                  | VA1-R              | CTGCCATTGATGCGCAAAAC    |                     |            |
|                  | m1                 | GTGCAAATGGCGATCG         |                     |            |
|                  | VA3-R              | CGTCTTGAATGGCGCAAAAC    |                     |            |
|                  | m2                 | GAGGCCCCAGGAAACATTTG    | 352-bp*             |            |
|                  | VA4-R              | CATACTAGCGCCTTGAC       |                     |            |

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Polymerase chain reaction

Amplification of \textit{cagA}, \textit{cagE}, and \textit{vacA} 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 \textit{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 \textit{cagA} and \textit{vacA} alleles are given in Table 2. Positive and negative reagent control reactions were performed with each batch of amplifications. DNA from \textit{H. pylori} strains ATCC 43504 (\textit{vacAs1a/m1}, \textit{cagA} positive), ATCC 51932 (\textit{vacA s2/m2}, \textit{cagA} negative), and ATCC 43526 (\textit{vacAs1b/m1}, \textit{cagA} positive) was used to define the accuracy of the \textit{cagA}. 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.

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. \textit{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...
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 cagA 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 cagAs1a/m1 was more frequently found in Pakistani H. pylori strains 39 (33%) compared with 17 (19%) (P = 0.025) in Afghan strains.

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

| Virulence marker | Pakistan n=119 (57) | Afghanistan n=91 (43) | P value |
|------------------|---------------------|-----------------------|---------|
|                  | Nonulcer dyspepsia  | Gastric ulcer n=12    | Gastric carcinoma n=20 | Duodenal ulcer n=20 |                  | 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)               |            |
| Vacs1/bm1        | 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)               |            |

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 = 0.053) 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, cagA 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 vacAs1a/b1 allele was present in 10 (59%) of GC compared with 20 (31%) in NUD (P = 0.033). The H. pylori genotype cagA/vacAs1a/m1
was associated in 8 (47%) with GC compared with 9 (14%) in NUD (P = 0.006) [Table 3]. The \textit{H. pylori} genotype \textit{cagA vacAs1a}/m1 was associated with GC in 7 (41%) compared with 7 (11%) in NUD (P = 0.007) [Figure 4 and Table 3].

In Pakistani \textit{H. pylori} strains, \textit{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]. \textit{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 \textit{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 \textit{H. pylori} \textit{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 \textit{H. pylori} genotype \textit{cagA vacAs1a}/m1 was associated in 13 (65%) with GC compared with 16 (24%) in NUD (P = 0.001).

**Correlation of histological changes with \textit{H. pylori} genotypes**

Marked gastritis was associated with \textit{cagA} among Afghans compared with Pakistanis patients [Table 4a]. \textit{CagE} was associated with neutrophil infiltration in both the groups [Table 4a]. Lymphocyte aggregate was significantly associated with \textit{cagA} in Pakistanis compared with Afghan patients. The distribution of \textit{vacA} alleles was not different among Afghan and Pakistani \textit{H. pylori} strains except that \textit{vacAs1b}/m1 and \textit{vacAs1b}/m1 were significantly distributed among Afghan \textit{H. pylori} strains compared with Pakistani \textit{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 \textit{H. pylori} strains were associated with NUD in majority of the patients. The density of \textit{H. pylori} strains did not vary in the two groups but Pakistani \textit{H. pylori} strains exceeded in their association with moderately active inflammation and lymphocyte aggregate formation compared with Afghan strains. The distribution of virulence markers \textit{cagA}, \textit{cagE}, and \textit{vacA} alleles were similar in the Afghan and Pakistan \textit{H. pylori} strains and \textit{H. pylori} \textit{cagE} was associated strongly with GC and DU compared with NUD, respectively. There was a difference in the \textit{vacA} signal “s” and middle “m” region types between Afghan and Pakistani \textit{H. pylori} strains. Among the Afghan strains, \textit{vacA} genotypes \textit{s1a} and \textit{m1} did not show association with peptic ulcer and GC compared with Pakistani strain. However, \textit{vacAs1b}/m1 allele in Afghan \textit{H. pylori} strains was associated with GC. In comparison, \textit{vacA} alleles “s1a” and “m1” were significantly associated with GC and peptic ulcer, respectively, compared with NUD in Pakistan strains. \textit{H. pylori} \textit{cag/s1a}/m1 was significantly associated with GC in Pakistani and GU in Afghan \textit{H. pylori} strains.

In an earlier study, genotypes of \textit{H. pylori} isolates obtained from 15 Afghan immigrants in Iran, the \textit{cagA} was positive in 60% and \textit{cagE} in 55% of Afghan isolates, while the most common \textit{vacA} s-region genotype was \textit{s1} in 80% and the \textit{s1/m1} was observed in 53%.[20] However, there was no significant association found between \textit{cagA}, \textit{cagE}, and \textit{vacA} genotypes and clinical outcomes in Iranian and Pakistani patients.
Afghan patients. 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 0.44% of total deaths. 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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