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Gastric lymphoma: association with *Helicobacter pylori* outer membrane protein Q (HopQ) and cytotoxic-pathogenicity activity island (CPAI) genes

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SUMMARY

B-cell non-Hodgkin lymphoma (B-cell NHL) is the second commonest malignancy in the stomach. We determined the distribution of *Helicobacter pylori* outer membrane protein Q (HopQ) allelic type, cytotoxin-associated gene (cag)-pathogenicity activity island (cag-PAI) and vacuolation activating cytotoxin A (*vacA*) genes, respectively, in patients with B-cell NHL. We also compared them with their distribution in non-ulcer dyspepsia (NUD). *H. pylori* was cultured from gastric biopsy tissue obtained at endoscopy. Polymerase chain reaction was performed. Of 170 patients enrolled, 114 (63%) had NUD and 56 (37%) had B-cell NHL. HopQ type 1 was positive in 66 (58%) in NUD compared with 46 (82%) ($P = 0{\cdot}002$) in B-cell NHL; HopQ type 2 was positive in 93 (82%) with NUD compared with 56 (100%) ($P < 0{\cdot}001$) in B-cell NHL. Multiple HopQ types were present in 46 (40%) in NUD compared with 46 (82%) ($P < 0{\cdot}001$) in B-cell NHL. CagA was positive in 48 (42%) in NUD *vs.* 50 (89%) ($P < 0{\cdot}001$) in B-cell NHL; cagT was positive in 35 (31%) in NUD *vs.* 45 (80%) ($P < 0{\cdot}001$) in B-cell NHL; left end of the *cagA* gene (LEC)1 was positive in 23 (20%) in NUD *vs.* 43 (77%) ($P < 0{\cdot}001$) in B-cell NHL. VacAs1am1 positive in B-cell NHL in 48 (86%) ($P < 0{\cdot}001$) *vs.* 50 (44%) in NUD, while s1am2 was positive in 20 (17%) in NUD *vs.* 46 (82%) ($P < 0{\cdot}001$) in B-cell NHL. *H. pylori* strains with multiple HopQ allelic types, truncated cag-PAI evidenced by expression of cagA, cagT and cag LEC with virulent vacAs1 alleles are associated with B-cell NHL development.

Key words: B-cell NHL, CagPAI, *Helicobacter pylori*, HopQ types1 and 2, vacA.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a common pathogen associated with human gastric and extragastric diseases. The failure of the host immune response to clear this infection results in chronicity. *H. pylori* infection is associated with the gastro-duodenal diseases that include chronic gastritis, peptic ulcer, i.e. gastric and duodenal ulcer, gastric carcinoma (GC) and mucosa-associated lymphoid tissue lymphoma (MALToma) [1]. *H. pylori* infection elicits an acute-phase response that activates an immune host response causing an imbalance between cell proliferation and cell apoptosis [2]. The gastric colonization

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of *H. pylori* depends on several factors that include urease enzyme, cytotoxin-associated geneA (*cagA*), vacuolating cytotoxin A (*vacA*) and *H. pylori* outer membrane proteins [3]. The genetic diversity among *H. pylori* strains accounts for varying manifestations among persons colonized with *H. pylori* [4]. The virulence of *H. pylori* is determined by markers, e.g. the cytotoxin-associated gene pathogenicity island (cag-PAI), vacA alleles, outer immunoproteinA and membrane protein Q (HopQ) type [5–9].

The genetic diversity in *H. pylori* is associated with high mutation rate [10, 11]. Infection with multiple strains promotes recombination between them. Genes encoding *H. pylori* outer membrane proteins are significant among the imported DNA fragments [10]. *H. pylori* genome sequences possess a large family of Hop genes [12]. *H. pylori* strains expressing HopQ have facilitated attachment to gastric epithelial cells [13]. Previously, we studied 241 *H. pylori* strains that showed HopQ type 1 in 70 (29%), type 2 in 60 (25%), and types 1 and 2 in 111 (46%) strains, respectively [5].

H. pylori infection predates the development of gastric lymphoma [6]. Previously, an association between *H. pylori* and gastric low-grade B-cell non-Hodgkin lymphoma (B-cell NHL) was described [14]. *H. pylori* was less common in high-grade gastric B-cell NHL in 38–51% with no specific mucosa-associated lymphoid tumor (MALT) features [6, 14]. *H. pylori* infection causes a chronic immuno-inflammatory reaction. This antigenic stimulation leads to lymphoid hyperplasia and acquisition of genetic instability, which follows activation of intracellular pathways [15]. The disease progresses with cellular proliferation, resistance to apoptosis and emergence of a malignant clone [15].

Ours is a low-risk region for GC with a high prevalence of *H. pylori* infection [16]. In a retrospective study that looked at the GC over a period of 10 years, 42 (11%) were cases of B-cell NHL [16]. In the current study, we determined the distribution of *H. pylori* and its virulence marker, i.e. cagA, cagAP, cagE, cagT, LEC, vacA alleles, and HopQ types 1 and 2 in B-cell NHL and compared their distribution with *H. pylori* strains associated with non-ulcer dyspepsia (NUD) and chronic gastritis.

MATERIAL AND METHODS

Patients

One hundred and seventy patients with upper gastrointestinal symptom that included abdominal pain were enrolled from the endoscopy unit extending from November 2012 to June 2016. Their mean age was 48 ± 13 years and range 21–83, male:female ratio was 1·4:1. Ours is a tertiary care center where the healthcare facilities are being availed by patients from all over the country. The hospital is located in a cosmopolitan city with an urban population of over 16 618 million in 2015. Population has varying ethnicity contributed by all the provinces of the country. Socio-economic status (SES) of majority of our patients varies from low to middle SES. One hundred and fourteen (67%) were diagnosed as NUD and in 56 (33%) B-cell NHLs. Other NHL subtypes, e.g. follicular, mantle, etc. were not found in this cohort of patients. Institutional ethics committee approved the study. Informed consent was obtained from all patients. The patients enrolled were not on any medications, such as antibiotics, H2-receptor antagonists, proton pump inhibitors, bismuth compounds in the last 3 months. A note was made of presenting symptoms and endoscopic findings (Table 1). Two gastric biopsies for histology were collected in formalin and four in normal saline two each for *H. pylori* culture and polymerase chain reaction (PCR). The PCR for *HopQ* alleles identified in *H. pylori* were single (i.e. type 1 or type 2) or multiple types (i.e. type 1 and type 2). *CagA*, *cagA*-promoter region (cagAP), cagE, cagT, left end of the *cagA* gene (LEC) and *vacA* alleles, i.e. s1a, s1b, m1, m2 and s2 were analyzed. Patients with *H. pylori* infection were treated with Bismuth-based quadruple therapy, while patients diagnosed with B-cell NHL were referred to oncologist for further management.

H. pylori culture

Each specimen was homogenized in eppendorf tubes with electric homogenizer and inoculated onto Columbia blood agar (Oxoid) medium supplemented with Dents supplement (containing trimethoprim, vancomycin, amphotericin B and cefsulodin) and 7% defibrinated sheep blood and incubated at 37 °C under microaerophilic conditions using anaerobic jars and strips (Campygen strips, Oxoid, UK) for 5 days producing microaerophilic conditions essential for the growth. Plates were then examined for bacterial growth. The identity of *H. pylori* was confirmed by colony morphology, Gram stain and production of urease and catalase. *H. pylori* isolates were defined as Gram-negative spiral-shaped bacilli that were urease and catalase positive.

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Table 1. *Comparison of patients with non-ulcer dyspepsia and non-Hodgkin B cell lymphoma*

	Non-ulcer dyspepsia ($n = 114$)	Non-Hodgkin B-cell lymphoma ($n = 56$)	*P* value
Age (years)			
Mean ± S.D.	42 ± 11	59 ± 5	
20–45 years	72 (63)	0 (0)	<0·001
46–79 years	42 (37)	56 (100)	
Gender			
Male	61 (53)	37 (66)	0·119
Female	53 (47)	19 (34)	
Symptoms			
Abdominal pain	114 (100)	44 (79)	<0·001
Weight loss	0 (0)	11 (20)	
Anorexia	0 (0)	1 (2)	
Histology			
Helicobacter pylori			
Positive	70 (61)	19 (44)	0·001
Negative	44 (39)	37 (56)	
Type of gastritis			
Chronic active gastritis	108 (95)	36 (64)	<0·001
Chronic gastritis	6 (5)	20 (36)	
Lymphoid aggregates			
Positive	40 (35)	56 (100)	<0·001
Negative	74 (65)	0 (0)	
PCR			
Positive	94 (82)	49 (88)	0·398
Negative	20 (18)	7 (13)	
HopQ type 1			
Positive	66 (58)	46 (82)	0·002
Negative	48 (42)	10 (18)	
HopQ type 2			
Positive	93 (82)	56 (100)	<0·001
Negative	21 (18)	0 (0)	
HopQ type			
Single type	69 (60)	10 (18)	<0·001
Multiple type	45 (40)	46 (82)	

Histology

An expert pathologist, unaware of the clinical details, reviewed all the biopsies and reported the diagnosis. All cases were shown to be of B-cell phenotype by immunochemistry with a panel of antibodies that included CD20, CD19, CD79a, CD22 and CD3. Diagnosis of B-cell NHLs was based on a positive CD20, CD19, CD79a and CD22· [17]. The criteria used for the diagnosis of B-cell NHL included diffuse sheets of large, blastic lymphoid cells, two to four times larger than normal lymphocytes, often infiltrating and destroying the gastric glandular architecture. Criteria for diagnosis of low-grade lymphoma of MALT type were as defined consisting of a diffuse proliferation of cells with epithelium infiltration forming characteristic lymphoepithelial lesions [18]. High-grade tumors were diffuse infiltrates of large blast cells [19]. Following endoscopy patients underwent whole-body computerized tomographic scan to determine the extent of involvement. Patients were referred to oncology service for further management. There were 15 cases of low-grade and 41 of high-grade B-cell NHL. Paraffin-coated gastric tissues were stained with hematoxylin and eosin to study histopathology and *H. pylori* [20].

Extraction of genomic DNA

DNA was extracted from gastric biopsy tissue as previously described [21].

PCR

Amplification of 16S rRNA, cagA, cagAP, cagE, cag LEC and cagT and vacA alleles by PCR was

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performed by the method previously used before [22] (Table 2).

HopQ genotyping

The HopQ type 1 and type 2 were determined by PCR methods described previously [23] (Table 2).

Sequencing of PCR product and BLAST Query

Sequence analysis was carried out by Macrogen (Seoul, South Korea). HopQ type 1 and type 2 sequences were published in our previous study and deposited in GenBank with accession numbers (KJ946296-KJ946308) and (KJ946309-KJ946314), respectively [5].

Sample size

The study determined the distribution of *HopQ* types in patients with B-cell NHL. From previous studies, *H. pylori* was present in 46% B-cell NHL [24] *HopQ* type 1 in GC in 53% and type 2 isolates in NUD in 41% [5]. Gastric ulcers were associated with *H. pylori* infection with multiple *HopQ* alleles in 46% compared with 23% with *HopQ* type 1. Therefore, the frequency of 46% with 95% level of confidence and (0·074) 7% bound on the error of estimation, a sample size of 175 patients was required. A total of 134 patients were required to establish their association with gastritis with 80% power. A sample size of 175 patients was required to cover both the study objectives.

Statistical analysis

Pearson χ^2, Fisher exact or likelihood ratio test were used where appropriate. A P value of <0·05 was significant. Data were analyzed using the SPSS version 19.0.

RESULTS

Patients with NUD were significantly younger than with B-cell NHL (Table 1). Abdominal pain was also significantly common in patients with NUD (Table 1). Endoscopically, there was gastritis in 114 (100%) NUD patients, while in B-cell NHL, there was a mass lesion 51 (91%) ($P < 0·001$). On histology, *H. pylori* was positive in 70 (61%) with NUD compared with 19 (34%) ($P = 0·001$) with B-cell NHL (Table 1). Lymphoid aggregates were common in B-cell NHL 56 (100%) compared with 40 (35%) ($P < 0·001$) in NUD (Table 1). Majority of B-cell NHL were diffuse large B cell that were high grade 41 (73%) with 30 out of 41 having >90% proliferative index (Table 3).

HopQ allelic type

HopQ type 1 was positive in 66 (58%) in NUD compared with 46 (82%) ($P = 0·002$) in B-cell NHL; while HopQ type 2 was positive in 93 (82%) with NUD compared with 56 (100%) ($P < 0·001$) in B-cell NHL (Table 1). Single HopQ allele was present in 69 (60%) with NUD compared with 10 (18%) in B-cell NHL, while multiple HopQ was present in 46 (40%) in NUD compared to 46 (82%) ($P < 0·001$) in B-cell NHL (Table 1).

Cag-PAI gene

CagA was positive in 48 (42%) in NUD compared with 50 (89%) ($P < 0·001$) in B-cell NHL; cagT was positive in 35 (31%) in NUD compared with 45 (80%) ($P < 0·001$) in B-cell NHL; cag LEC1 was positive in 23 (20%) in NUD compared with 43 (77%) ($P < 0·001$) in B-cell NHL; while cag LEC2 was positive in 7 (6%) in NUD compared with 27 (48%) ($P < 0·001$) in B-cell NHL (Table 4).

VacA alleles

VacAs1am1 was positive in B-cell NHL in 48 (86%) ($P < 0·001$) compared with 50 (44%) in NUD, while s1bm1 was positive in 31 (55%) ($P < 0·001$) in B-cell NHL compared with 19 (17%) in NUD; s1am2 was positive in 20 (17%) in NUD compared with 46 (82%) ($P < 0·001$) in B-cell NHL; while s1bm2 was positive in 2 (2%) in NUD and in 33 (59%) ($P < 0·001$) in B-cell NHL (Table 4).

DISCUSSION

PCR for the *H. pylori* 16S rRNA PCR did not demonstrate any difference in the association of *H. pylori* with NUD and B-cell NHL (Table 3). This is particularly likely if *H. pylori* tested on the biopsy obtained from the B-cell NHL involved area, which may be necrotic and has low *H. pylori* load. PCR for *H. pylori* is known to have a higher yield [25]. Chronic active gastritis was predominant in both groups (Table 1). Infiltration of the gastric mucosa with neutrophils and lymphocytes leads to increased apoptosis and

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Table 2. *Primers used in PCR experiments*

Region amplified	Primer designation	Primer sequence (5′ to 3′)	Size of PCR product	PCR cycles
16Sr RNA	C97	GTCATGACGGGTATCC	1200 bp	One cycle of 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min; 72 °C for 5 min
	C98	ACTTCACCCCAGTCGCTG		
cagA	D008	GGTCAAAATGCGGTCATGG	297 bp	One cycle of 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 90 s, one cycle of 72 °C for 5 min
	R008	TTAGAATAATCAACAAACATCACGCCAT		
cagAP	cagAP-F1	GTGGGTAAAAATGTGAATCG	730 bps	One cycle of 94 °C for 5′; 35 cycles of 1 min at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min
	cagAP-R1	CTGCAAAAGATTGTTTGGCAGA		
*cag*E	cag E- F1	GCGATTGTTATTGTGCTTGTAG	329 bps	
cagE-R1		GAAGTGGTTAAAAAATCAATGCCCC		
*cag*T	cagT-F1	CCATGTTTATACGCCTGTGT	301 bps	
cagT-R1		CATCACCACACCCTTTTGAT		
LEC1	LEC-F1	ACATTTTGGCTAAATAAACGCTG	384 bps	
LEC-R1		TCTCCATGTTGCCATTATGCT		
LEC2	LEC-F2	ATAGCGTTTTGTGCATAGAA	877 bps	
LEC-R2		ATCTTTAGTCTCTTTAGCTT		
HopQ				
Type 1 HopQ, region A	OP5136a	CAACGATAATGGCACAAACT	524 bps	One cycle of 95 °C for 5 min, 30 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, one cycle of 72 °C for 5 min
	OP4829b	GTCGTATCAATAACAGAAGTTG		
Type 1 HopQ, region B	OP4070c	CAATTCCCCTGCCTATCAAGCC	372 bps	
	BA8705d	TGATGTGGTTACATGCGCTTC		
Type 2 HopQ				
	BA8363e	TCCAATCCAGAAGCGATTAA	430 bps	
	BA8364f	GTTTTAATGGTTACTTCCACC		
Vac alleles S1a	SS1-F	GTCAGCATCACACCGCAAC	190-bp	One 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; one cycle of 72 °C for 5 min
	VA1-R	CTGCTTGAATGCGCCAAAC		
S1b	SS3-F	AGCGCCATACCGCAAGAG	187-bp	
	VA1-R	CTGCTTGAATGCGCCAAAC		
M1	VA3-F	GGTCAAAATGCGGTCATGG	190-bp	
	VA3-R	CCATTGGTACCTGTAGAAAC		
M2	VA4-F	GGAGCCCCAGGAAACATTG	352-bp	
	VA4-R	CATAACTAGCGCCTTGCAC		
S2	VA1-R	CTGCTTGAATGCGCCAAAC	286-bp	
	VA1-F	ATGGAAATACAACAAACACAC		

Table 3. *Distribution of non-Hodgkin B-cell lymphoma*

	Grade of lymphoma		
	Low grade $n = 15$ (27)	High grade $n = 41$ (73)	*P* value
Helicobacter pylori on histology			
Positive	10 (67)	9 (22)	0·002
Negative	5 (33)	32 (78)	
16S PCR of *H. pylori*			
Positive	12 (80%)	16 (39)	0·014
Negative	3 (20%)	25 (61)	
Ki-67			
>90%	3 (20)	30 (73)	<0·001
>80%	8 (53)	10 (25)	
>70%	4 (27)	1 (2)	

n (%), number and percentage.

clearance of epithelial cell. *H. pylori*-induced macrophages and dendritic cells (DCs) that are known to secrete tumor necrosis factor-α that further promote inflammation [26]. After activation via Toll-like receptors, DCs are known to activate T cells to Th1 or Th2 response by expression of interleukin (IL)-12 or IL-10, respectively [27]. Dendritic cells exposed to *H. pylori* for 48 h exhibited a markedly attenuated ability to induce interferon-γ production that contributed to the persistence of the infection [28].

HopQ has a role in the pathogenesis of gastric B-cell NHL as it promotes attachment of the *H. pylori* to the epithelial cells. *H. pylori* binds to host cell receptors via adhesion molecule of the carcinoembryonic antigen (CEACAM) located on the gastric epithelial cell membrane to translocate cagA into host cells [29]. Multiple types of HopQ types were associated with B-cell NHL compared with the NUD (Table 1). In patients with B-cell NHL, both HopQ type 1 and HopQ type 2 were present and a multiplicity of the HopQ types was demonstrated in 82% of B-cell NHL (Table 1). In an animal study, naïve B cell exposed to *H. pylori* demonstrated a biphasic response in which low multiplicity of infection (MOI) (1–10) induced cellular proliferation and markedly inhibited apoptosis [30, 31]. Low levels of *H. pylori* infection that occur in vivo are associated with B-cell survival and proliferation, consistent with their potential to evolve into MALToma. The difference in the clinical outcome was not only due to the longer duration of the *H. pylori* infection in the B-cell NHL but was also contributed to by the virulence of the infecting *H. pylori* strains.

Table 4. *Comparison of Helicobacter pylori virulence groups marker in different*

	Non-ulcer dyspepsia ($n = 114$)	Non-Hodgkin B-cell lymphoma ($n = 56$)	*P* value
CagA			
Positive	48 (42)	50 (89)	<0·001
Negative	66 (58)	6 (11)	
CagA-promoter			
Positive	34 (30)	20 (36)	0·438
Negative	80 (70)	36 (64)	
CagE			
Positive	43 (38)	17 (30)	0·345
Negative	71 (62)	39 (70)	
CagT			
Positive	35 (31)	45 (80)	<0·001
Negative	79 (69)	11 (20)	
CagLEC1			
Positive	23 (20)	43 (77)	<0·001
Negative	91 (80)	13 (23)	
CagLEC2			
Positive	7 (6)	27 (48)	<0·001
Negative	107 (94)	29 (52)	
VacAs1am1			
Positive	50 (44)	48 (86)	<0·001
Negative	64 (56)	8 (14)	
VacAs1bm1			
Positive	19 (17)	31 (55)	<0·001
Negative	95 (83)	25 (45)	
VacAs1am2			
Positive	20 (17)	46 (82)	<0·001
Negative	94 (83)	10 (18)	
VacAs1bm2			
Positive	2 (2)	33 (59)	<0·001
Negative	112 (98)	23 (41)	
VacAs2m1			
Positive	3 (3)	5 (9)	0·117
Negative	111 (97)	51 (91)	
VacAs2m2			
Positive	28 (25)	7 (12)	0·068
Negative	86 (75)	49 (88)	

n (%), number and percentage.

The limitation of this study is that we did not do immunohistochemical (IHC) staining to detect the genetic aberrations such as t(11;18)(q21;q21) and t(1;14)(p22;q32) that are associated with *H. pylori*-independent B-cell NHL [32]. For patients without t(11;18)(q21;q21) or t(1;14)(p22;q32), nuclear translocation of BCL10 and nuclear factor-κB (NF-κB) detected by IHC is predictive of *H. pylori*-independent state [33]. CagA expression in tumor cells, particularly with nuclear expression is a useful biomarker in lymphoma cells, and is associated with the direct

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lymphomagenic effect of *H. pylori* on B cells. The titers of anti-*H. pylori* and anti-cagA antibodies were not checked. These titers have been reported to be significantly higher in *H. pylori*-dependent cases than in *H. pylori*-independent cases of t(11;18)(q21; q21)-negative gastric MALT lymphoma [34, 35]. In the presence of cagA, B-cell lymphocytes evade apoptosis through the inhibition of p53 accumulation [36, 37]. An important limitation of this study is that lymphoma patients are older than non-lymphoma patients, raising the possibility that lymphoma is a consequence of duration of infection, age, or some other factor, apart from *H. pylori* pathogenicity factors. The high mutation rates of *H. pylori* and chronic infection in the lymphoma patients may involve strains lacking the pathogenic markers. The cross-sectional nature of this study cannot account for the duration of infection with more or less pathogenic strains. However, these limitations do not invalidate the study.

H. pylori cagA was significantly associated with B-cell NHL (Table 4). This is consistent with a previously reported *cagA* 78–100% association with B-cell NHL [31]. CagPAI genes, i.e. *cagA promoter* and *cagE* genes, were not significantly associated with either of the two conditions (Table 4). This variability may be attributed to DNA motifs that exhibited sequence heterogeneity in the *cagA* gene [38]. The cagPAI in B-cell NHL appears to be partially truncated as cagA promoter was 20 (36%) and *cagE* 17 (30%), respectively, compared with cagT and cagA LEC (Table 4). In an earlier local study, the presence of the *cagA* did not signify an intact cagPAI [39]. Most of the *H. pylori* strains studied had partial cagPAI with missing *cagE* and *cagAP*s [39]. *CagA*-positive *H. pylori* strains are potent in induction of host inflammatory responses, including activation of neutrophils, which releases highly genotoxic oxygen reactive species that induces barrier dysfunction and apoptosis in the gastric epithelium. CagA activation of the NF-κB affects cell proliferation through c-Fos and c-Jun and impaired immune response by inducing apoptosis of T cells [40]. In our study, *cagT* and *LEC* genes were significantly associated with B-cell NHL (Table 4). CagE is required for the induction of IL-8 by host cells [41] and is a component of the *H. pylori* Type 4 Secretion System (T4SS) [42]. It was also equally common in the *H. pylori* strains associated with NUD and B-cell NHL (Table 4). The *cagT* gene encoded an extracellular lipoprotein of the T4SS complex that stabilizes the other proteins [42]. *H. pylori* also induces IL-8 via T4SS constituent cagL interaction with the host receptor integrin b1 and the subsequent activation of the mitogen-activated protein kinases and NF-kB pathway [43].

VacA s1 alleles were significantly associated with B-cell NHL compared with NUD (Table 4). Both *vacA*s1/m1 and s1/m2 are virulent form common in gastric diseases [44]. VacAs1/m2 has been variably reported about its association with MALToma [14, 45]. The underlying mechanisms involve *vacA* blocking antigen presentation to T cells [46], T-lymphocyte activation [47] and maturation of macrophage phagosomes [48], thus suppressing T-cell responses to *H. pylori* and contributing to the immunosuppression and chronicity of *H. pylori* infection [49]. Immune cells that recognize and attack *H. pylori* accumulate near the site of infection but are ineffective in eliminating the bacterium.

In conclusion, *H. pylori* infection with multiple HopQ types, truncated cagPAI with increased expression of cagT, LEC and vacAs1 alleles are associated with B-cell NHL in our patients. IHC will be useful in these cases to look for the genetic aberrations associated with *H. pylori*-independent B-cell NHL.

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AUTHOR CONTRIBUTIONS

Study concept and design – J.Y., Z.A.; acquisition of data – J.Y., Z.u.A., K.M.; analysis and interpretation of data – J.Y., Z.A., K.T., S.A., Z.u.A.; drafting of the manuscript – J.Y., Z.A., S.A., R.K.; critical revision of the manuscript for important intellectual content – J.Y., Z.A., S.A., Z.u.A., R.K., K.M.; statistical analysis – J.Y., S.A., Z.A.; obtained funding – J.Y.; administrative, technical or material support – K.T., J.Y., K.M.; study supervision – R.K., J.Y., K.T.

DECLARATION OF INTEREST

None.

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