Review article

Helicobacter pylori in Iran: A systematic review on the association of genotypes and gastroduodenal diseases

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Background: Helicobacter pylori (*H. pylori*) infection is known as a major etiologic factor for a variety of gastroduodenal diseases. In Iran, with a high rate of *H. pylori* infection close to 90%, numerous studies have revealed many aspects of interaction between the bacterium, mucosal surface and induction of disease outcome. The organism is genetically diverse and several virulence factors are attributed to the more virulent strains. The well-characterized virulence factors of *H. pylori* are cytotoxin associated gene A and vacuolating cytotoxin gene A. The distribution pattern of *H. pylori* genotypes and its association with disease status varies geographically. The present review focused on the virulence factors and genotyping of *H. pylori* in relation to gastroduodenal disorders in different regions of Iran. **Methods:** In total, 398 studies were reported on different aspects related to *H. pylori* in our electronic search from 1995-2011. *H. pylori* infection and its virulence factors in association with disease status were investigated in 159 reports. Looking specifically at the gastrointestinal tract disorders, the most relevant reports including 37 papers were selected. **Results:** We found no correlation of cagA genotype and disease status in the majority of studies, whereas vacA was demonstrated as a useful marker in predicting the disease outcome. The results of reports on other virulence factors of *H. pylori* such as blood group antigen-binding adhesion gene A, the induced by contact with epithelium gene A, the outer inflammatory protein A, the duodenal ulcer promoting gene A, and *Helicobacter* outer membrane gene and their relation with disease status were contradictory. **Conclusions:** Although different markers of *H. pylori* were emphasized as useful when predicting disease outcomes in some studies, the inconsistent researches and the scarcity of data made any conclusion or even comparison impossible. Considering the gap of information observed during our search relating to genotyping and other aspects of *H. pylori* infection, further investigations are suggested.

Key words: *H. pylori*, Genotyping, Virulence, Gastroduodenal, cagA, vacA, Iran

INTRODUCTION

Helicobacter pylori (*H. pylori*) - gram-negative, spiral-shaped, microaerophilic bacterium is one of the most prevalent bacterial infections throughout the world affecting half of the world population.[1] The distribution pattern of *H. pylori* infection ranges from 25-50% in developed countries to more than 80% in the developing world.[2] Low socioeconomic status and education level, crowded living places in childhood, and poor oral hygiene are among risk factors for carrying *H. pylori*.[3-5] It has been implicated as a major player in several gastrointestinal diseases as well as a number of extra gastric related disorders.[6-8]

H. pylori is genetically diverse and several virulence factors have been attributed to the more virulent strains. The presence of flagella, which allows the bacterium to move and find a more livable environment within the stomach, and the ability to produce ammonia from urea using its urease enzyme are two certain capabilities of *H. pylori* to survive the harsh environment of the stomach.[7] The most studied virulence markers of *H. pylori* include a cytotoxin-associated gene pathogenicity-island (cag PAI), a vacuolating cytotoxin gene A (vacA), and a blood group of antigen-binding adhesion gene A (babA).

In Iran, *H. pylori* infection is present in nearly 90% of adult population[9] and appears to occur early in life, with > 50% of children infected before age 15.[10] Besides the fact that *H. pylori* was introduced as a class I carcinogen,[11] the infection is difficult to cure and requires various combination therapies.[12] On the other hand, a high rate of antibiotic resistance for various antimicrobials is considered as the major cause of the *H. pylori* treatment failure.[13] The low eradication rate[14] and a considerable reinfection rate (20%)[15] indicate the significance of

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controlling *H. pylori* infection as an important health problem in Iran. Diagnostic methods, pathogenesis of *H. pylori* towards various diseases, treatment strategies and evaluation of bacterial resistance against some antimicrobials leading to treatment failure, have been all the matters of interest for numerous studies performed in Iran. The present review however, has focused on virulence factors and genotyping of *H. pylori* in relation to gastroduodenal disorders for which we found no comprehensive review in Iranian literatures.

Methods

To conduct a systematic review, the primary search was performed and the terms *"Helicobacter pylori*" as title and "Iran" as address were looked up within the time-span of 1995-2011. We performed our search using the Web of Knowledge database where the most relevant studies in the fields of microbiology and gastroenterology were indexed as ISI (Institute for Scientific Information). In total, 398 studies were reported on different aspects related to *H. pylori* in our electronic search. Based on title screening, we categorized all the papers into six major categories including virulence factors, treatment and therapy strategies, antimicrobial resistances, disease associations, and diagnostic methods. The other aspects such as immunological, biotechnological, and pharmacological researches were categorized as "others". *H. pylori* infection and its virulence factors in association with disease status were investigated in 159 reports. Looking specifically at the gastrointestinal tract disorders, the most relevant reports including 37 papers were selected. Other publications related to *H. pylori* infection and extra digestive disorders such as Down syndrome, metabolic syndrome, and anemia were excluded. We included the relevant original and review articles and abstracts indexed as ISI and all the selected papers were fully reviewed. Hand searching, we also included the references of the selected articles indexed as ISI or found in Google Scholar database as well as those published in national Iranian journals (in English) and not indexed as ISI since 1995. In our study, *H. pylori* infection and gastrointestinal tract pathogenesis along with genotyping and disease association were discussed as two main sections in details.

H. pylori infection and gastrointestinal tract pathogenesis

Chronic gastritis and peptic ulcer disease (PUD)

H. pylori colonization is known as the main cause of gastritis and PUD.[9,16] The frequencies of gastritis and PUD among Iranian patients with *H. pylori* infection are reported to range from 25-79% and 11-62%, respectively. In a study by Sotoudeh et al.,[17] 508 participants were enrolled in a national population-based endoscopic survey. Mucosal biopsies were obtained from different sites of the gastrointestinal tract. The number of polymorphic nuclear leukocytes (PMN) and mononuclear (MN) cells for chronic gastritis were assessed. Both PMN and MN infiltrations correlated strongly with *H. pylori* infection being maximum for the antrum (odds ratio (OR) = 9.4) and the minimum for the gastric body (OR = 1.7).[17]

Atrophy, intestinal metaplasia (IM), and dysplasia

Chronic gastritis causes repeated and ongoing injury and destruction of the epithelial cells. In some patients this progresses to mucosal atrophy, IM and dysplasia, eventually ending in gastric cancer (GC).[17] The histopathological findings in particular atrophy and IM indicated no correlation with *H. pylori* virulence factors in the first-degree relatives of GC patients.[18] However, an increasing risk of GC was revealed due to the correlation between histological findings, as pre-neoplastic changes, and virulence factors of 207 *H. pylori* infected patients in another study.[19]

Gastric cancer (GC)

H. pylori infection to be a cause of GC was firstly suggested by Warren.[20] GC is the fourth most common malignancy in the world and *H. pylori* infection has been attributed to over 63% of all cases of GC worldwide.[21] In Iran, the high rate of *H. pylori* infection, ranging from 42- 89%, has been reported for the areas with higher incidence of GC (the northern and northwestern regions).[22] In Ardabil, northwest of Iran, a province with high incidence of GC,[23] the prevalence of *H. pylori* infection has been estimated as 82.2% in the study population. Chronic gastritis was also noticed in more than 90% of subjects. Moreover, atrophy, IM, and dysplasia were seen in 71.8% of subjects indicating the significant correlation of gastric precancerous lesions with *H. pylori* infection in this study.[22] A significant correlation was also related to higher *H. pylori* infection rates for GC mortality in both men and women in Babol, the Caspian Sea coast, Iran.[24] A meta-analysis of prospective studies by Danesh suggested that GC is 2-3 times more common in those infected by *H. pylori*.[25]

Esophageal cancer

There is evidence that *H. pylori* infection has protective effect against gastroesophageal cancer. A meta-analysis by Islami and Kamangar, using all the searched publications from the PubMed and ISI databases,

suggested a consistent inverse association between esophageal cancer and *H. pylori* infection.[26] The protective effect of *H. pylori* infection against esophageal cancer was further confirmed by the same authors in a review paper about *H. pylori* and its effects on human health and disease.[7]

Gastroesophageal reflux disease (GERD)

As for esophageal cancer, an inverse association of *H. pylori* with GERD has also been documented. A neuro-immunological mechanism is responsible for the protective effect of *H. pylori* infection against GERD.[27] The role of *H. pylori* infection in development of GERD was investigated in 263 Iranian children. The prevalence of *H. pylori* infection among patients with GERD (13/83, 15%) was significantly lower than in those without GERD (46/180, 26%) ($p < 0.05$).[28] However, in a case-control study, patients with GERD (case group) were compared with normal gastrointestinal endoscopic findings (normal group). The rate of *H. pylori* infection was insignificantly lower in the case group compared to the control (81.5% vs. 87.1%, $p = 0.29$).[29]

Other gastrointestinal tract diseases

Recurrent abdominal pain (RAP) is defined as the occurrence of at least three episodes of several abdominal pain affecting activities over a period longer than three months. *H. pylori* infection, as a possible cause of RAP, was investigated in 40 children with RAP (mean age = 12.7 ± 1.0 years) and 60 healthy children as controls. A similar prevalence of *H. pylori* infection was found in patients with RAP compared to the control group ($p = 0.112$).[30]

The role of *H. pylori* infection in upper gastrointestinal (UGI) bleeding was investigated in persons with hemophilia (PWH). Prevalence of 76.7% and 55% of *H. pylori* infection in patients with and without a history of UGI bleeding was considered as an important cause of UGI bleeding in PWH ($p = 0.03$).[31]

The presence of *H. pylori* infection in dental plaque showed no significant association in patients with and without gastric infection ($p = 0.6$).[32]

Genotyping and disease association

The predisposition of *H. pylori* to cause diseases has been attributed to its diverse genome. Several virulent genes have been identified to contribute to the pathogenicity and severity of these diseases.[33-35] In the following section, the most vastly studied virulence factors including cag PAI, vacA, babA, the induced by contact with epithelium gene A (iceA), the outer inflammatory protein A (oipA), the duodenal ulcer promoting gene (dupA), and the *Helicobacter* outer membrane gene (hom), in relation to various gastroduodenal diseases are discussed. We reviewed some aspects of these genotyping including frequencies, pathogenicity towards more prevalent gastroduodenal manifestations, structural variability, and co-existences in details. Table 1 is a summary of genotyping distribution pattern of *H. pylori* in association with clinical outcomes in Iranian patients.

Cytotoxin-associated gene pathogenicity-island (cag PAI)

The cag PAI, a well established genotype of *H. pylori*, is a 40-kb region located in the chromosomal glutamate racemase gene. Its 35% G+C content distinguishes it from the rest of the bacterial genome with a 39% G+C content.[34] The cag PAI contains 31 genes of which 6 encode type IV secretion system mediating bacterial factors into the gastric epithelial cells.[36] *H. pylori* strains are classified as type I and II based on whether or not they possess the cag PAI. While type II strains lack functional cag PAI, type I strains possess the cag PAI region and induce more pathogenicity than the former strains.[34,37,38] Cytotoxin associated gene A and E (cagA and cagE) are two representative genes of the cag PAI and are associated with gastritis, PUD and gastric adenocarcinoma. The cagA gene is located in the right half of the cag PAI and has two ends of a very conserved 5′ and a variable 3′. It affects the epithelial cells by inducing cellular signaling and elicits a number of cellular changes, proliferation, and apoptosis.[39] The cagE is located upstream of cagA and is involved in the process of interleukin 8 (IL-8) expressions.

Simultaneous detection of cagA and cagE were assessed in relation to various gastroduodenal outcomes. Biopsy specimens were taken and the genes were detected using gene specific polymerase chain reaction (PCR). Although only cagA-positive strains were associated with GC, a higher prevalence of cagE strains and identical distribution pattern in PUD and GC patients with cagA (Table 1) indicated the potency of cagE as a better virulence marker.[40] In another study, cagA prevalence (71%) in 128 *H. pylori* infected patients correlated well with PUD in Rasht, north of Iran (Table 1).[41] However, no correlations were found between cagA genotype and gastritis, PUD, and GC in biopsy specimens obtained from 60 male and 40 female patients in Isfahan, central Iran (Table 1).[42]

As discussed previously, the 3′ region of cagA gene in *H. pylori* is highly variable. Depending on the types

Table 1. Genotyping distribution pattern of H. pylori isolates in association with clinical outcomes in Iranian patients

Genotyping	Hp+ isolates No.	NUD (%)	PUD* (%)	Gastritis (%)	GC (%)	Overall distribution n (%)	City	Ref.
cag A^+ **	120	78[a]	94[ab]	ND ***	100[b]	101(84)	Tehran	40
cag E^+		88	94	ND	100	109(91)	Exp.	(2009)
cag A^+ **	107	ND	78[a]	46[b]	ND	76(71)	Rasht Exp.	41 (2009)
cag A^+	100	ND	72	65	60	68(68)	Isfahan Exp.	42 (2008)
cag A^+	235	89	96	ND	94	213(91)	Tehran Exp.	46 (2008)
cag A^+	231	ND	54	70	62	154(67)	Tehran	49
cag E^+			44	39	12	90(39)	Exp.	(2009)
cag T^+			37	29	25	70(30)		
cag A^+/cag E^+/cag T^+			19	17	0	40(17)		
cag A^-/cag E^-/cag T^-			27	19	25	47(20)		
cag A^+	80	80	70	ND	ND	62(77)	Tehran	50
cag E^+		35	35			28(35)	Exp.	(2008)
cag T^+		36	25			27(34)		
s1 **	80	72[a]	58[ab]	ND	100[c]	61(76)	Tehran	55
s2		28	42		0	19(23)	Exp.	(2010)
m1 **		24[a]	25		61[b]	26(32)		
m2		76	75		39	54(67)		
s1m1 **		24[a]	25[ab]		61[c]	26(32)		
s1m2		46	33		39	35(44)		
s2m2		28	42		0	19(24)		
s1 **	132	68[a]	79[b]	ND	ND	93(71)	Tehran	58
s2		31	14			36(27)	Exp.	(2003)
m1		33	27			42(33)		
m2		55	59			74(55)		
s1m1		28	21			35(27)		
s1m2 **		28[a]	49[b]			43(33)		
s2m1		4	0			4(3)		
s2m2		27	10			31(23)		
s1	178	NA****	NA	NA	NA	143 (81)	Shahrekord	57
s2		NA	NA	NA	NA	35 (20)	Exp.	(2009)
m1		NA	NA	NA	NA	129 (73)		
m2		NA	NA	NA	NA	49 (27)		
s1m1		11	67	NA	NA	36(20)		
s1m2 **		3[a]	58[b]	NA	NA	96(54)		
s2m1		NA	NA	NA	NA	7(4)		
s2m2		NA	NA	NA	NA	39(22)		
cag A^+ **	137	46	35[a]	ND	67[b]	61(44)	Tehran	59
s1		77	79		78	107(78)	Exp.	(2005)
s2		23	21		22	30 (22)		
m1		13	17		39	25 (18)		
m2		28	26		55	54 (39)		
s1m1		6	7		5	9 (6)		
s1m2		43	15		22	39 (28)		
s2m1		3	2		0	3 (2)		
s2m2		2	2		0	2 (1)		
cag A^+	77	ND	79	48	ND	52(67)	Rasht	60
s1			81	55		55(71)	Exp.	(2009)
s2			NA	44		22(29)		
m1 **			65[a]	0[b]		31(40)		
m2			NA	100		29(38)		
cag A^+	96	74	79	ND	100	73(76)	Tehran	61
s1		72	53		100	66(69)	Exp.	(2008)
s2		27	42		0	27(28)		
m1		35	21		0	30(31)		
m2		58	68		100	59(61)		
s1m1		27	11		0	22(23)		
s1m2		41	37		100	40(42)		
s2m1		8	11		0	8(8)		
s2m2		18	32		0	19(20)		

Genotyping	Hp+ isolates No.	NUD (%)	PUD* (%)	Gastritis (%)	GC (%)	Overall distribution n (%)	City	Ref.
cag A$^+$	126	NA	NA	ND	NA	93 (74)	Tehran	63
s1		NA	NA		NA	80 (63)	Exp.	(2006)
m2		NA	NA		NA	77 (61)		
s1m2 **		33[a]	NA		54[b]	47 (37)		
cag A$^+$*****	74	72	46	ND	25	48(65)	Tehran	66
cag E$^+$		52	33		25	32(47)	Exp.	(2009)
s1		67	62		100	50(68)		
s2		33	38		0	24(32)		
m1		26	46		25	22(30)		
m2		74	54		75	52(70)		
s1m1		21	15		25	15(20)		
s1m2		46	46		75	35(47)		
s2m1		5	31		0	7(9)		
s2m2		28	8		0	17(23)		
cag A$^+$ *****	55	74	62	ND	64	37(67)	Tehran	67
s1		NA	NA	NA	NA	37(67)	Exp.	(2010)
m2		NA	NA	NA	NA	38(69)		
s1m2		NA	NA	NA	NA	26(47)		
cagA$^+$ *****	59	76	76	ND	ND	45 (76)	Tehran	68
s1m1i1		NA	NA			15 (25)	Exp.	(2008)
s1m1i2		NA	NA			1 (2)		
s1m2i1		NA	NA			4 (7)		
s1m2i2		NA	NA			16 (27)		
s2m2i2		NA	NA			16 (27)		
cagA$^+$	207	NA	NA	ND	NA	194(94)	Tehran	19
s1 **		NA[a]	NA		94[b]	159(77)	Exp.	(2009)
s2		NA	NA		NA	48(23)		
m1		NA	NA		NA	67(32)		
m2		NA	NA		NA	138(66)		
i1 **		44[a]	59[ab]		88[c]	110(53)		
i2		NA	NA		NA	94(45)		
s1m1i1		NA	NA		NA	64(31)		
s1m1i2		NA	NA		NA	5(2)		
s1m2i1		NA	NA		NA	47(23)		
s1m2i2		NA	NA		NA	43(21)		
s2m2i2		NA	NA		NA	48(23)		
babA2 **	81	ND	74	68[a]	80[b]	58 (72)	Isfahan	72
							Exp.	(2010)
babA	72	48	33	ND	75	NA	Tehran	74
babB		77	92		94		Exp.	(2008)
dupA	157	ND	45	50	46	78 (50)	Tehran	79
							Exp.	(2008)
cagA$^+$	138	ND	55	51	68	80 (58)	Sari	83
homA **			79[a]	57[b]	15[c]	75 (54)	Exp.	(2011)
homB **			21[a]	43[b]	78[c]	60 (43)		

Abbreviations: Hp$^+$, *H. pylori* positive; ND, not determined; Exp, experimental study; NA, not available
* PUD was considered as the frequency of genotyping in GU plus DU subjects if data available; otherwise the values are for either GU or DU subjects separately.
** Significant correlation of specific genotype with clinical outcomes (The significant differences are shown with different letters a, b, and c, wherever present.)
*** ND: The specific clinical outcome was not examined in the respective study.
**** NA: The missing values which were not found or could not be calculated from the respective study.
***** The values presented in the table are only for Persian subjects examined, as other ethnicities also were compared in the respective studies.

and number of repeat regions including R1 (15 bp), R2 (42 bp) and R3 (147 bp), four types (motifs) of A, B, C, and D are contributed to the 3′ end variability of cagA. The types are distinguished by the PCR product size ranging from 642-651 bp for A type, 756 bp for type B and type D, and 813-815 bp for type C.[43] Salehi et al. used the CAG1 and CAG2 primers to amplify the 3′ region of the cagA gene. They investigated the subtypes of 3′ region of cagA gene in *H. pylori* strains and their relation to gastroduodenal diseases.[41] As determined by the analysis of the molecular size of the PCR products,[43] three subtypes of A, B, and D and no C type were detected.[41] A larger number of type C phosphorylation motifs has been reported to be more pathogenic.[44] Accordingly, the authors indicated no association of 3′ region subtypes with clinical

outcomes examined.[41] In a similar approach, 3′ end variable region of the cagA gene was analyzed and its relation with non-ulcer dyspepsia (NUD), PUD, and GC was studied.[45] While 94% of the strains were type ABC, 3.3% had four copies (ABCC type), and 3.3% had two copies (AB type). Further analysis of deduced protein sequences confirmed that there was no detection of type D sequences in the studied population. Moreover, no relation with any of the diseases was found.[45]

In another study, the frequency of cagA in *H. pylori* strains was assessed by means of genotyping and serological assessment of host antibodies.[46] Single biopsy sampling resulted in 90.6% of cagA-positive strains with no correlation to clinical outcomes (Table 1). Multiple biopsy sampling in this study resulted in 78.4% of strains harboring cagA gene. The $cagA^+/cagA^-$ strains contributed to 16.5% of the results. Still, this result confirmed the more suitability of multiple biopsy sampling compared to single sampling in predicting the genotyping frequencies as indicated by others.[47] Furthermore, the presence of anti-CagA antibodies showed an association with clinical manifestations when categorized in three groups of NUD, PUD, and GC. PUD and GC occurrence were strongly associated with the presence of anti-CagA antibodies and no anti-CagA sero-negative cases were found in patients with these two diseases. Considering the similar frequency of anti-CagA antibodies and cagA genotyping (90.7% and 90.6%, respectively) and their association with clinical outcomes, serum reflection of cagA genotypes was found to be a suitable approach in predicting disease outcome in patients at high risk.[46]

While cagA and cagE are located more downstream of the cag I region in the cag PAI, the cagT gene is located in the upstream cag II region and reported to be related to severe clinical outcomes.[48] Pathogenicity of the cag PAI was determined by analysis of the latter three genes by Baghaei et al.[49] Neither single genes nor combination of cag I and cag II regions (cagA, cagE, and cagT) were found to be correlated with gastroduodenal diseases (Table 1). Although there was no relation between cagA status and disease in this study, higher prevalence of cagA gene in different clinical isolates was thought to be a better marker than cagE for determining disease association (Table 1).[49] In a similar study, a lower prevalence of cagT and a higher frequency of cagA in PUD patients again indicated the importance of cagA as a virulence marker. They concluded that the presence of both cag I and cag II regions of the cag PAI was not necessary for developing ulcer disease.[50]

Vacuolating cytotoxin gene A (vacA) and its co-existence with cagA genotype

The vacA, one of the vastly studied virulence determinants of *H. pylori*, has a mosaic structure comprising three families of allelic variation including the signal sequence region (s1, s2), the mid region (m1, m2), and the recently discovered intermediate region (i1, i2), located between the signal and middle regions of the vacA genotype.[51] Inducing cytoplasmic vacuolation in epithelial cells,[52] inducing apoptosis through cytochrome release from mitochondria,[53] suppression of epithelial proliferation and migration, and inducing cytoskeletal changes[54] are attributed to the cytotoxin activity of the vacA gene (protein).[51,55]

Applying PCR-based methodology, different combinations of allelic variations such as s1m1, s1m2, and s2m2 were detected from dyspeptic patients who underwent upper gastrointestinal endoscopy. Cytotoxic activity of vacA gene seemed to be attributed to these different combinations. The vacA genotyping revealed a strong association between the s1 and s1m1 genotypes and GC when compared to NUD and PUD in Iranian patients (Table 1).[55] This association was further confirmed assessing the vacuolating activity of different genotypes using cultured HeLa cell monolayers. Interestingly, s1m1 type protein was capable of producing prominent cell vacuolation, whereas s2m2 lacked the ability.[55] The vacA s1 and s1m1 genotypes were also the dominant genotypes among Iranian patients with high rate (90%) of *H. pylori* infection in another study.[56] However, the collective assessment of the vacA genotype signal sequence and middle region determined a majority of s1m2 genotype in patients with PUD which was significantly correlated with the disease ($p < 0.05$) in two other studies (Table 1).[57,58]

The first study to demonstrate the frequency of cagA and vacA genotypes was performed in 2005 in Iran by Siavoshi et al. who obtained gastric biopsy specimens from 137 patients to examine the association between mixed strain genotyping and clinical outcomes. Cancer occurred more frequently in patients infected with $cagA^+$. In addition, vacA s1 was the most common genotype in patients with PUD (Table 1). However, due to a very small sample size of cancer patients (18/137) and the fact that there was no significant association between $cagA^+$ or vacA s1 genotypes with PUD, cagA and vacA genotypes were not considered

as helpful in predicting disease outcome in this study.[59] Similarly, the co-existence of vacA subtypes and cagA were studied in relation to clinical outcomes from biopsy samples taken from patients in Rasht, north of Iran. The genotype s1m1/cagA$^+$ was the most frequent genotype among patients with PUD. In gastritis patients, most of the strains were s2m2/cagA$^-$ genotype. The vacA s1 and m1 were found as predominant in patients with PUD, whereas all patients with gastritis had m2 allele (Table 1).[60]

In another study by Jafari et al., irrespective to clinical outcomes (NUD, PUD, and GC), vacA s1m2/cagA$^+$ genotype was predominant in strains isolated from Tehran, Iran.[61] As with vacA genotypes, the prevalence of the cagA gene was not correlated with any clinical outcomes. The presence of cagA was significantly associated with the vacA s1 genotype ($p < 0.001$). They also found an 8% prevalence for s2m1 genotype which has been reported to be rare in Iranian populations.[55] Yet, vacA s1m2 and cagA$^+$ strains correlated well with gastroduodenal diseases in other studies.[62,63]

The geographical variation in *H. pylori* genotypes and its link to the severity of gastroduodenal disease were previously reported.[64,65] In Iran, in addition to Persian and Azeri Turks as the major ethnic groups, four other distinct ethnic groups including Kurds, Lurs, Arabs, and Afghans, predominate. Distribution of *H. pylori* cagA, cagE, and vacA subtypes were determined by PCR to compare different ethnic groups living for a long time in Tehran, Iran.[66] The cagA was found in 65%, 73%, and 71% of isolates from Persians, Turks, and other ethnicities (as one group), respectively. No correlation was found between cagA status and clinical manifestations including NUD, PUD, and GC in any ethnic group ($p = 0.13$, $p = 0.76$, and $p = 1.0$, respectively). The cagE status was the highest among other ethnicities (Kurds, Lurs, Arabs, and Afghans) compared to Persians and Turks (77%, 47%, and 30%, respectively; $p = 0.008$). The predominant vacA genotypes were s1 and m1 in all three ethnic groups (Persians, Turks, and other ethnicities). As for cagA, no correlation with clinical outcomes was found for cagE and vacA genotypes.[66] Consistently, no significant associations were found between cagA, cagE, and vacA genotypes and clinical outcomes (NUD, PUD, and GC) comparing Iranian and Afghanian isolates of *H. pylori* in another study.[67] In addition, the co-existence of triple positive genotype of cagA$^+$/cagE$^+$/vacA s1 indicated no association with NUD and PUD in any of two ethnicities.[67]

H. pylori infection is an important risk factor for GC, the second most important cause of cancer deaths worldwide.[25] A higher incidence of GC in Iran was hypothesized to be due to the differences in virulence markers between *H. pylori* strains from Iran and those from Iraq.[68] In fact, a similar frequency of cagA genotype was seen in both isolates from Iran and Iraq (77% and 71%, respectively) with association to PUD ($p < 0.01$) only in strains from Iraq. Although no significant association between vacA alleles and PUD existed among Iranian strains, vacA i1 was associated with PUD in strains from Iraq ($p < 0.02$). Moreover, cagA presented more motifs (ABCC) in Iranian strains compared to Iraqi strains (ABC). The cagA gene with more phosphorylation motifs could be contributed to the higher incidence of GC in Iran. Nonetheless, other host and environmental factors were considered to be more important in GC pathogenesis in the two countries.[68]

Rhead et al. identified the third polymorphic determinant of vacuolating activity, i-region, by nucleotide sequencing and allele specific PCR. Using a simple PCR-based typing system, the association of i-region with GC was examined in 73 Iranian patients who underwent endoscopy. Strains with s1m1 subtype were predominantly i1 and s2m2 strains were all i2. However, the i-region varied in s1m2 subtypes. Moreover, vacuolating activity varied between the strains depending on various subtypes. While all strains with s1m1i1 genotype showed vacuolating activity and strains with s2m2i2 were nonvacuolating, strains with s1m2 genotype varied and only the s1m2 strains possessing i1 showed the vacuolating activity. GC was associated with vacA s1 allele ($p < 0.05$), cagA ($p < 0.005$), and m1 allele ($p < 0.05$) compared to the NUD group as control. Interestingly, vacA i-type strain was also significantly correlated with GC ($p < 0.005$). Previous studies indicated the s1m1 and s1m2 genotypes of vacA as suitable virulence markers. Yet, due to the fact that i-type included all pathogenic strains with s1m1 and those of s1m2 with vacuolating activity, i-type was considered as a sufficient marker for disease pathogenesis.[51]

Similar to many previous studies, Douraghi et al. assessed clustering of active virulent genes in association with NUD, PUD, and GC in 207 *H. pylori* infected patients. They also studied vacA polymorphism in correlation with histopathological findings. Strains with s1 and s1m1i1 were related to GC ($p < 0.005$ and $p = 0.001$, respectively), whereas s2m2i2 genotype was correlated with NUD.

Multivariate logistic regression analysis found i-region superior to s and m regions of the vacA gene. Possession of s1i1/cagA+ genotype was an independent predictor of metaplasia (OR = 3), dysplasia (OR = 9.9) and risk of GC (OR = 6.9).[19] However, vacA alleles and cagA revealed no association with the types of gastritis with or without atrophy or IM in the first-degree relatives of GC patients.[18]

H. pylori has long been a strict human stomach pathogen. Yet, the reservoir of this pathogen and its mode of transmission are unclear. A study on dental plaque of 100 patients with dyspepsia was performed in Tehran, Iran. *H. pylori* infection was found in 40% of samples of which 62.5% were cagA-positive. Accordingly, dental plaque was estimated to be a suitable reservoir and a possible route of transmission for *H. pylori*.[69] In another study, vacA genotypes isolated from stomach and saliva were compared in 250 patients undergoing upper gastrointestinal tract endoscopy in Shahrekord, Iran and 189 (75.6%) were found to be *H. pylori* positive. The vacA analysis was performed for 36 patients whose saliva and gastric samples were both positive for *H. pylori*. A high homology (61%) was obtained in vacA genotypes between stomach and saliva. Still, the diversity of 38.3% in *H. pylori* strains between two sites and a high rate of *H. pylori* infection (75.6%), firstly suggested more than one strain to possibly exist in stomach and saliva of the same patient, and secondly estimated saliva as a suitable reservoir for *H. pylori* infection.[70]]

babA

The gene product is a member of family of *H. pylori* outer membrane proteins. It mediates the adherence of *H. pylori* to the Lewis blood group antigens on human gastric epithelial cells.[71] Three bab alleles have been identified as babA1, babA2, and babB. The first two ones are identical except that babA1 has a 10-bp deletion of the translational initiation codon. Therefore, only babA2 product is necessary for binding activity.[71] Relation of babA2 genotype with chronic gastritis, PUD, and non-cardia gastric cancer was examined in Isfahan, Iran.[72] The frequency of babA2 was 58 (71.6%) in 81 *H. pylori* positive specimens. There was no correlation between genotype and clinical outcomes when gastritis and PUD were compared ($p = 0.673$), but a significant correlation was observed comparing gastritis and non-cardia gastric cancer ($p < 0.001$) (Table 1).[72] This association was further confirmed in another study in which a 40.6% frequency of babA2 genotype was significantly associated with GC compared to NUD and PUD patients ($p = 0.0004$).[73] Relationship between babA and babB genotypes and disease outcome in another study showed a higher frequency of babA limited to GC patients, whereas babB was higher among GC and PUD subjects (Table 1).[74]

iceA

The iceA is a signaling gene, induced by contact with epithelium. There are two allelic types of the gene: iceA1 and iceA2. The products of two allelic variants are different and the expression of iceA1 is up-regulated following contact between *H. pylori* and human epithelial cells.[8] The prevalence of iceA genotypes in 75 *H. pylori* strains isolated from PUD patients was investigated in Sari, north of Iran. The iceA1 and iceA2 genotypes verification was determined via PCR. The iceA1 genotype (64%) was more prevalent than iceA2 (21.3%). A significant correlation between iceA1 genotypes and PUD occurrence ($p = 0.03$) indicated that iceA1 gene can be used as a reliable marker in predicting the clinical outcomes of *H. pylori* infection.[75] In another study, iceA genotypes were determined in 30 *H. pylori* positive isolates using PCR. Both alleles, iceA1 and iceA2, were found in 66.7% and 23.8% of the isolates, respectively. The presence of two strains (9.5%) with no iceA1 and iceA2 genotypes seemed to show the presence of a new allele other than iceA1 and iceA2 alleles in *H. pylori*.[76]

oipA

The oipA is an important virulence factor associated with enhanced IL-8 secretion and increased inflammation in vitro as well as clinical outcomes.[77] It encodes one of the outer membrane proteins and is an inflammation-related gene located approximately 100 kb from the cag PAI on the *H. pylori* chromosome.[77] A study of various *H. pylori* virulence factors compared this gene in different major ethnic groups in Tehran, Iran.[66] The prevalence of oipA was found to be 51%, 33%, and 71% in Persians, Turks, and other ethnicities (Kurds, Lurs, Arabs, and Afghans), respectively. No correlation with clinical outcomes was found for any ethnicity. In Persians, it was more common in NUD (63%) than in PUD (15%) or GC patients (0%) ($p = 0.001$).[66]

dupA

The dupA is a recently described virulence factor, comprising the jhp0917 and jhp0918 genes which form one continuous gene. Lu et al. noticed a significant role of dupA in development of duodenal ulcer (DU) which was related to neutrophil infiltration and a high level of IL-8 production by epithelial cells. On the other hand, possession of this gene appeared to be protective

against gastric adenocarcinoma.[78] The presence of the jhp0917 and jhp0918 genes was assessed in 157 *H. pylori* infected patients with DU, gastric ulcer (GU), gastritis, and GC using gene specific PCR. After the tests, 78 (49.7%) strains were positive for both, the jhp0917 and jhp0918, genes. Possession of dupA was not a promoting or protective determinant for any of clinical outcomes. However, the presence of dupA gene was inversely associated with dysplasia ($p < 0.05$) as the final stage towards GC and 83.3% of dysplasia-positive strains were colonized with dupA-negative strains. Moreover, dupA indicated inverse association with lymphoid follicles as a consequence or relatively long-term infection by *H. pylori*.[79]

hom

The gene products constitute a small paralogous family of proteins that contain alternating hydrophobic motifs and signal sequences in the C terminus which are typical of other outer membrane proteins.[80] The homA and homB are the most studied members of the hom family and are 90% identical. These genes vary in the central domain where six different allelic variants within a 300-bp region exist. It has been suggested that the number of homB genes affects the number of bacteria adhering to host cells and that the presence of homB is associated with secretion of the pro-inflammatory cytokine IL-8, PUD, and GC.[81,82] The prevalence of virulence genes homA, homB, and cagA in a population at very high risk of cancer was assessed in the north of Iran (Table 1). The cagA genotype had no significant impact on disease state ($p = 0.2654$). The homA and homB genotypes indicated significant association with disease status in any comparison stratification (Table 1). The co-existence of cagA and homA was inversely associated ($r = -0.279$; $p = 0.001$), whereas a significant positive association was found between cagA and homB ($r = 0.243$; $p = 0.004$). In this population, the high prevalence of homB among GC patients (78%) indicated that homB may be a better predictor of more virulent strains of *H. pylori* and influence the severity of disease manifestation.[83]

DISCUSSION

In total, including six duplicates, 398 studies have been reported in various research areas relating to *H. pylori* infection in Iran since 1995. One can query these studies in the web of Knowledge database using the terms "*Helicobacter pylori*" as title and "Iran" as address in the time-span of 1995-2011. As for other countries,[64,65] we found the importance of knowledge on the geographical variation in *H. pylori* genotypes and its link to the severity of gastroduodenal disorders reported in Iran to figure out the further investigations. We focused on the virulence factors of *H. pylori* and their associations with disease status as many studies have demonstrated the importance of *H. pylori* virulence factors in disease progression.

The cagA, as a marker for the presence of the cluster of genes (cag PAI), is one of the best studied virulence markers of *H. pylori*. The frequency of cagA-positive isolates has been reported to be nearly 100% in some countries in East Asia such as Japan, China, and Korea, 60-80% in some other countries such as Taiwan, Turkey, Malaysia, India, and Bangladesh, and 25-60% in Bahrain, Israel, and Jordan.[46] The cagA genotype varied geographically ranging from 44% to 94% in Iranian populations. A strong correlation between possessing cag PAI and its representative gene cagA and severe clinical outcomes has been reported in several studies.[37,38] We found a significant association of cagA genotype with chronic gastritis,[70] PUD,[50,84] and GC.[40,59,63] However, the majority of studies indicated no influence of cagA genotype on the progression of diseases.[18,42,46,49,61,62,66-68,83] Accordingly, in other Asian countries where the majority of *H. pylori* infected individuals harbor the cagA genotype, the association between this genotype and disease state was not observed.[85,86] The high prevalence of cagA genotype in Iran as well as East Asian countries on one hand,[46] and the number of studies illustrating no influence of this genotype on disease status on the other hand, questions the value of cagA genotype as a suitable marker when predicting the related clinical outcomes in this part of the world.[46] In Iran however, the majority of studies investigating the relation of cagA genotype with disease status have been performed in Tehran, the capital city, probably due to the more facilities and equipped laboratories (Table 1). Therefore, further investigations are needed in other areas to elucidate whether the cagA genotype could be a reliable marker for disease progression.

Another vastly studied virulence factor of *H. pylori* is the vacA gene. The variations in the three regions of vacA gene of *H. pylori* s, m, and i region are known to cause the differences in vacuolating activity. In a meta-analysis comparison, the prevalence of vacA alleles in single and in combinations varied significantly among different countries in the Middle East.[87] Countries in the northern parts such as Turkey and Iraq had a higher frequency of s1 allele (79.4%) compared to Jordan, Saudi Arabia, Kuwait, and Israel as southern countries (44.5%, $p < 0.001$).[87] Consistent with other countries in the northern part of the Middle East we

found a frequency of 72% for vacA s1 in our analysis. The vacA s1 was the most common genotype in Iranian populations followed by m2 and m1 alleles (55.3% and 36.1%, respectively). Comparing vacA genotypes in the Middle East, our findings were in a great consistence in particular for vacA m1 allele for which the frequency was estimated as 36.1% in neighbor countries such as Turkey and Iraq. However, vacA m1 genotype showed a much lower frequency in southern countries of the Middle East especially in Israel with a prevalence of only 8%. In the Middle East, the combination of vacA s and m genotypes indicated s1m2 as the predominant genotype (42.1%), followed by s1m1 (30.5%) and s2m2 (26.1%). The average frequency of vacA s2m1 was only 1.4% in the Middle East. Our findings indicated vacA s1m2 as the most common subtype of combined genotypes (40.7%), followed by s1m1 and s2m2 (21.3% and 18.8%, respectively) which is again in agreement with other regions in the Middle East.

Overall, unlike the cagA status, the majority of studies emphasized the implication of vacA polymorphic structure in increasing risk of gastroduodenal diseases. However, no association of vacA genotypes and disease status was found in some other studies.[18,61,67] Looking at the association between vacA genotypes and disease status, a significant association was observed between s1 genotype and gastritis,[62,70] PUD,[56-58,60,88] and GC.[19,51,55] The vacA m1 genotype indicated strong correlation with PUD[60] and GC,[55] whereas m2 genotype correlated with gastritis.[60] The vacA s1m1 genotype was correlated with GC,[55] whereas s1m2 allele indicated significant correlation with gastritis,[62,63,70] PUD,[57,58] and GC.[63] In our search, there were only two studies reporting the third region of vacA genotype, i subtype. Compared to the s and m regions, it was suggested that i region of the vacA genotype is a superior marker in predicting the results concerning the disease status.[19,51] Mixed polymorphic determinant of vacA genotype s1m1i1 indicated a significant prevalence in subjects with GC and IM, while s2m2i2 genotyping was most prevalent in NUD patients.[19] Co-existence of cagA and vacA genotypes, s1m1/cagA$^+$, s1m2/cagA$^+$, s1i1/cagA$^+$, and s1/cagA$^+$ were all correlated with disease status in some studies.[60-63] A strong correlation of vacA genotype with clinical outcomes in the majority of the studies implies that vacA in any polymorphic structure could still be considered as the best virulence marker when predicting disease outcomes.

Despite the vast investigation on cagA and vacA genotypes, there are considerably rare studies on the association of other *H. pylori* virulence factors and clinical outcomes in Iran. We found reports on the association of the adherence factor of babA2 and disease status, particularly GC.[72,73] The frequencies of babA2 allele varied from 40.6% (Sari, northern Iran) to 71.6% (Isfahan, central Iran) in these studies.[72,73] Another adherence factor, iceA, with two allelic types of iceA1 and iceA2, was studied in two cities of Babol and Sari, north of Iran.[75,76] A higher frequency was noticed for iceA1 genotype compared to iceA2 allele. Moreover, a significant two-tailed frequency correlated iceA1 with PUD compared to iceA2 allele in the study by Talebi et al.[75] There was no correlation for an inflammation-related gene oipA and disease status in different ethnicities living for a long time in Iran.[66] The frequency of 49.7% of dupA indicated no effect towards any of clinical outcomes, whereas it was inversely associated with precancerous lesions.[79] It was indicated that homB may be a better predictor of more virulent strains of *H. pylori* than homA, even though they were both correlated with the severity of disease manifestation. Although different markers of *H. pylori* were emphasized as useful when predicting disease outcomes in some studies, the inconsistent researches and the scarcity of data made any conclusion or even comparison impossible. Further studies are necessary to estimate the actual effect of these genotypes in Iranian populations. It is also important to determine whether other pathogenic factors such as *H. pylori* neutrophil-activating protein (Hp-NAP),[86] *H. pylori* arginase (rocF),[87] and *H. pylori* adhesion protein A (HpaA)[88] other than previously studied virulent factors could be useful in predicting the disease outcomes.

The discrepancy observed in the frequency of *H. pylori* genotypes and their association with disease state in Asian countries as well as developed countries[89] could not be explained precisely. Yet, several causes may contribute to the observed discrepancy: i) subject selection and the fact that in most of the studies subjects were recruited among patients referring to the health centers with symptomatic clinical outcomes and could not be then representative of general population; ii) lack of enough sample size with respect to clinical symptoms and genotyping; iii) the geographic origin of the patients (host) and bacterial and environmental factors; IV) the various detection methods of *H. pylori* in the first step and subsequent analysis of genotyping using PCR-based technology applying various primers and laboratory protocols; and V) type (single vs. multiple) and site of sampling which varied from cardia, corpus, and pylorus to the antrum in different studies.

Further suggestions

Overall, according to the search performed, the number of studies increased from 2 to 74 yearly, over the period (1995-2011). We observed a gap of information and a less tendency towards some aspects of *H. pylori* investigation before 2004. While there was no drastic change in any of the categories, there was more frequency towards all six categories after the year 2004. Interest to investigate the association of *H. pylori* with various diseases was increased over the period and became the main contributing factor in 2011. Treatment strategies were investigated during the whole period except in 1996 for which no study was found in any category. Antimicrobial resistance was rarely studied before 2004. Further on, it fluctuated over the time and gradually increased in the recent years. Antimicrobial resistance is important if it leads to a successful treatment of *H. pylori* infection.[90] A great geographical variation has been reported for bacterial resistance of *H. pylori* strains against various antimicrobials.[91,92] The co-investigation strategies would therefore be required to make us aware of the local antibacterial resistance rate and the subsequent appropriate regimen to eradicate *H. pylori* infection.

We found few papers over the period of our search estimating the actual prevalence of *H. pylori* infection among general population. Population-based studies were also found in other databases investigating the seroprevalence of *H. pylori* infection in a few geographical areas. Selecting sample size representative enough to general population, further research on the prevalence of *H. pylori* infection in different geographical areas would be another matter of interest. Considering a very high rate of *H. pylori* infection reported in a number of studies, effective public health interventions in some areas is necessary. Eventually, comprehensive research strategies for *H. pylori* infection from the very first step of childhood monitoring and diagnosis towards treatment strategies and the subsequent eradication evaluation for different geographical areas are suggested.

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