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Original Article

The Frequency of *Helicobacter pylori* Infection in Patients with Primary Antibody Deficiencies

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Abstract

Introduction: Primary antibody deficiencies (PADs) are the most common inherited immunodeficiencies, which can present wide clinical presentation including susceptibility to bacterial infections and gastric adenocarcinoma. Since *Helicopter pylori* (*H.pylori*) infection is associated with immune dysregulation and an increased risk of gastric carcinogenesis, we evaluated the prevalence of HP infection in patients with different forms of PAD.

Methods: Thirty-seven patients with common variable immunodeficiency (CVID), 23 patients with X-linked agammaglobulinemia (XLA), and eleven patients with hyper IgM syndrome (HIgM, age range 8-25; 47 males and 24 females) were screened for *H.pylori* infection by Urea breath test (UBT) and *H.pylori* stool antigen (HPSA). Subsequently, an upper gastrointestinal endoscopy was conducted only for patients who had UBT and HPSA positive results due to an established gastrointestinal indication.

Results: Although almost all patients were under prophylactic antibiotic therapy, *H.pylori* infection was detected in 28% (n=20) of the patients; among different forms of PAD, 29% (n=11) of CVID patients, 30% (n=7) of XLA, and 18% (n=2) of HIgM patients were infected. Among patients with *H.pylori* infection, the rate of parasite infections was higher, while the prevalence of autoimmunity and autoinflammatory disorders increased in patients without *H.pylori* infection.

Conclusions: Despite regular immunoglobulin replacement therapy and antibiotic prophylaxis, one-fourth of PAD patients had a persistent *H.pylori* infection though without severe gastrointestinal manifestations. Long-term follow-up of these selected patients is essential to evaluate its association with gastric cancers.

Keywords Disorder, Primary immunodeficiency, Helicopter pylori, Gastric cancer.

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Introduction

Primary immunodeficiency disorder (PID) refers to a heterogeneous group of over 350 disorders that result from defects in immune system development and/or function. Primary antibody deficiencies (PADs) include a wide spectrum of inherited disorders characterized by impaired specific antibody production and decreased serum immunoglobulin (Ig) levels (1). The most common symptomatic types of PAD disorders include common variable immunodeficiency (CVID), X-linked agammaglobulinemia (XLA), and hyper IgM syndrome (HIgM) (2).

PAD patients are commonly susceptible to recurrent infections particularly in the respiratory and gastrointestinal (GI) tracts; however, autoimmune diseases (e.g. inflammatory bowel disease, enteropathy and crohn's disease), and malignancy (e.g. gastric adenocarcinoma) are also seen in these patients (3-7). Altogether, GI manifestations are the second most prevalent complication of PAD that have been reported in approximately 10-50% of patients with different types of PADs (8, 9). Although an increased risk of gastric cancer in these patients suggests that genetic predisposition and environmental factors could be involved in the development of malignancy, the main cause still remains to be elucidated (6, 10, 11).

Helicobacter pylori (*H.pylori*) could affect 20-80% of the general population and is known as a significant risk factor for peptic ulcers and chronic active gastritis. Further, a correlation between *H.pylori* infection and atrophy with intestinal metaplasia in the stomach has been evident (12-14). On the other hand, *H.pylori* eradication in high-risk populations has diminished the relative risk of gastric cancer (15) and contributes to prevention of the progression of some precancerous gastric lesions (16, 17).

The aim of this study is to evaluate the frequency of *H.pylori* infection in PAD patients under regular Ig replacement therapy and antibiotic prophylaxis. We also compared the prevalence and the clinical symptoms of *H.pylori* infection in different forms of PADs.

Materials and methods

Patients

Among all symptomatic PAD patients registered in

the national PID database (2), all patients with regular follow-up and punctual treatment who were referred to Children's Medical Center (Pediatrics Center of Excellence affiliated to Tehran University of Medical Sciences, Tehran, Iran) were enrolled in this study during 2015-2016. The process of this study was approved by the ethics committee of Tehran University of Medical Science and written informed consents were also obtained from both the adult patients and the children's parents. The CVID, XLA, and HIgM diagnoses and classification were based on European Society for Immunodeficiencies (ESID) diagnostic criteria, and secondary causes of dysogammaglobulinemia were ruled out in all patients (https://esid.org/Working-Parties/Registry/Diagnosis-criteria).

Methods

All suitable volunteers underwent ^{14}C urea breath test (UBT) using the PYTEST kit and a microCOUNT scintillation counter (Ballard Medical Products, Draper, Utah, USA). The test results were categorized as follows: <50 disintegrations per minute (dpm) was defined as a negative (normal), ≥50 but <200 dpm was an indeterminate, and ≥200 dpm was *H. pylori* positive. Furthermore, a fresh stool sample for *H.pylori* stool antigen (HpSA) testing was provided and analyzed using the HPSA enzyme immunoassay kit according to the manufacturer's instruction (Meridian Diagnostics, Inc., Ohio, USA). The mean OD of negative control at 450nm, plus 0.1 was obtained according to manufacturer's recommendation (GA Generic Assay, Germany). Negative HPSA test was defiend by OD ≤ cut-off, while OD>cut-off was considered *H. pylori* positive. Patients with positive results for both tests (UBT and HPSA) were marked as *H. pylori* infected and subsequently were referred to a gastroenterologist to perform upper GI endoscopy for patients who had complete medical indications.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 software (Chicago, USA). Fisher's exact test and chi-square tests were used for 2 × 2 comparisons of categorical variables, while t-tests, one-way ANOVA, and their nonparametric equivalent were used to compare numerical variables. Shapiro-Wilks test was used to check the normality assumption for a variable.

Results

A total of 71 patients including 37 CVID patients, 23 XLA patients, and 11 HIgM patients (47 males [66.2%] and 24 females [33.8%]) with a median age at diagnosis of 7.0 (3.5-13) years were enrolled. The demographic and immunological characteristics of patients are summarized in **Table 1.** Among 71 patients, 55 (77.4%) patients had positive C^{14}-UBT results, while HPSA test was positive for only 20 (28%) cases. Accordingly, 11 (29 %) CVID patients, 7 (30%) patients with XLA, and 2 (18%) patients

with HIgM were marked as *H. pylori* positive infection.

Note that UBT is a highly sensitive and specific diagnostic method for *H. pylori* infection; for assessing whether the organism has been successfully eradicated following antimicrobial therapy, achlorhydria was suspected to occur with a high false-positive rate of in 35 patients. However, the usage of proton pump inhibitors (PPIs) did not differ between *H. pylori* infected and non-infected patients with positive UBT (2/20[10%] vs. 4/35[11.4%]). Further, pernicious anemia was not recorded in any patient in this study, suggesting infection with other gastric spiral Urease-positive organisms. The rete of UBT positive/HPSA negative patients was similar in three different PAD groups: 51.3% of CVID patients, 43.4% of XLA patients, and 45.4% in HIgM patients.

Table 1. Demographic and immunological data of primary antibody deficient patients

Parameters	Total	CVID	XLA	HIgM
Sex ratio (M/F)	47/24	16/21	23/0	8/3
Age at the study time, year (IQR)	18.0(8-25)	21.7 (10-29)	13 (5-12)	12 (3-22)
Age at diagnosis of PAD, year (IQR)	7.0(3.5-13)	10 (5-15.5)	5 (4-6)	4.5 (2.5-6.5)
Diagnosis delay, year (IQR)	3.0(1-6.0)	4.5(2-7)	2.5(1-3.5)	2(0.5-4)
Consanguinity, Number (%)	43 (60.5)	24(67.8)	12(52.1)	7(63.6)
IgG, mg/dl (IQR)	180(39-443)	161(50-341)	20(6-42)	134(51-381)
IgA, mg/dl (IQR)	5(0-13)	15(5-26)	0.9(1-6)	8(2-20)
IgM, mg/dl (IQR)	19(5.5-64)	19.5(12.3-38.2)	5(2-10.3)	135(114.6-235)
IgE, IU/mL (IQR)	1(0-5)	1(0-8)	2(0-3)	1(0-5)
Lymphocytes, cell/ul (IQR)	1979(140-3550)	2200(1350-3720)	1940(1120-2410)	1638(1090-2940)
CD3+ % of lymphocytes (IQR)	79(69-87)	76.6(60-80.2)	86.7(75.8-90.6)	70.5(66.2-81.3)
CD4+ T cells, % of lymphocytes (IQR)	35(23-43)	30.5(28.7-37.4)	41(34.2-46)	37.5(32-40.6)
$CD8^+$ T cells, % of lymphocytes (IQR)	41(30-50)	41.8(29.7-48.2)	41.5(30-45.8)	34.9(28.5-37.5)
$CD19^+$ % of lymphocytes (IQR)	6.4(0-13)	8(4-12.5)	0.5(0-1.5)	9(6.3-14.4)

IQR: 25^{th} to 75^{th} inter quintile range

Our results revealed that *H. pylori* positive patients tended to be older than *H. pylori* negative patients (20 [11-28] vs. 16 [8-24] years) and there was a significant difference in the duration of diagnosis delays between these two groups (P=0.01). In addition, there was a significant reduction in serum levels of IgA in *H. pylori* positive patients (1[0-7]) compared with those without *H. pylori* infection (7[019], P=0.07, **Table 2**). Notably, use of effective antibiotics against *H. pylori* (clarithromycin, amoxycillin, cefotaxime, and ciprofloxacin)

during the two months prior to the study time was not different between infected and non-infected patients (4/14 and 7/20, respectively).

Table 2. Characteristics of immunoglobuline levels in the primary antibody deficient patients

Number	characteristic	HP-patients [n=51(71%)]	HP+ patients [n=20(28%)]	P value
1	Total IgG mg / dl	160(35-441)	200(49-495)	0.7
2	Total IgM mg / dl	17(7.5-67)	19(5-64)	0.6
3	Total IgA mg / dl	7(0-19)	1(0-7)	0.07
4	Total IgE mg /dl	1(0-11)	1(0-2)	1

Table 3 reports the detailed clinical presentations and histological features of *H. pylori* positive patients. Among the patients with *H. pylori* infection, only two CVID patients had a gastrointestinal indication for endoscopy. Chronic active gastritis was observed in one of the patients with *H. pylori* infection (P2) who manifested involvement of both antrum and fundus in the endoscopy, while another CVID patient with an indication for endoscopy indicated a normal result after endoscopy (P1).

Regarding the gastrointestinal symptoms, statistical analysis showed that the prevalence of diarrhea was higher in *H. pylori* positive patients compared with those without infection (0.19 [0.05-0.21] vs. 0.06 [0-0.09]/year, *P*=0.1), though this difference was not significant. Interestingly, the rate of parasitic infections (e.g. *Blastocystis hominis* and *Giardia lamblia*) was significantly higher in patients with *H. pylori* infection (6/20 [30%]) compared to other patients (1/51 [1.9%], *P*=0.001). Further, the autoinflammatory disorders and autoimmunity were significantly frequent in the patients without *H. pylori* infection (22/51[43.1%] vs. 3/20[15%], *P*=0.02). There was no significant correlation between the incidence of other related gastrointestinal symptoms, including chronic diarrhea (13/20 [65%] vs. 24/51[47%], *P*=0.19), epigastric pain (2/20 [10%] vs. 5/51[9.8%], *P*=0.65), vomiting (1/20 [5%] vs. 4/51[7.8%], *P*=0.70), weight loss (2/20 [10%] vs. 4/51[7.8%], *P*=0.72), and irritable bowel syndrome (IBS, 1/20 [5%] vs. 2/51[3.9%], *P*=0.9) with diarrhea.

Discussion

Inflammatory and infectious GI disorders are present in 10-50% of patients with PID (8, 11). Previous studies have suggested that *H. pylori* infection could be associated with GI disorders in CVID patients (especially gastritis); however, the underlying mechanisms are not fully understood (11, 12, 18). Accordingly, we examined the GI symptoms and the prevalence of *H. pylori* infection in CVID, XLA, and HIgM patients.

In the present study, *H. pylori* infection was seen in 28% of our early onset PAD patients' cohort (74.6% of patients were tested during their childhood at the time of study).

Table 3. Clinical and immunological characteristics of *H. pylori* positive primary antibody deficient patients

ID	PAD	DOO (y)	DOD (y)	DD (y)	Age of study	Sex	Cons	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	CD3 (% of lymphocytes)	CD4 (% of lymphocytes)	CD8 (% of lymphocytes)	CD19 (% of lymphocytes)	Clinical Manifestation
p1	CVID	4	10	6	27	M	1	190	0	84	74%	18%	55%	6%	Otitis, sinusitis, conjunctivitis, skin involvement, arteritis, diarrhea, epigastric pain,
p2	CVID	28	28	0	38	F	0					51%			Diarrhea, Celiac Marsh II, Antral type mucosa with severe chronic active gastritis
p3	CVID	4	16	12	30	F	0	1023	1	2	89%	37%	66%	8%	Pneumonia, sinusitis, LAP, splenomegaly, diarrhea, epigastric pain, colitis,
p4	CVID	1	10	9	19	F	1	433	15	19	63%	26%	29%	17.4%	Pneumonia, bronchiectasis, diarrhea,
p5	CVID	8	16	8	25	M	1	0	17	6	89%	32%	44%	3.7%	Pneumonia, sinusitis, arteritis, LAP, bronchiectasis, diarrhea,
p6	CVID	11	14	3	18	M	0	495	7	0	76%	29%	46%	15.8%	Pneumonia, diarrhea,
p7	CVID	36	36	0.7	40	M	0	161	0	34	86%	37%	49%	9.4%	Pneumonia, sinusitis, splenomegaly, diarrhea,
p8	CVID	1	10	9	29	M	1	284	41	9	83%	23%	56%	7%	Pneumonia, sinusitis, otitis, skin involvement, arteritis, bronchiectasis, diarrhea, IBS
p9	CVID	0.5	34	33.5	41	M	0	0	0	42	80%	19%	62%	4%	Pneumonia, sinusitis, otitis, arteritis, diarrhea
p10	CVID	7	26	19	28	F	0	442	5	91	76%	43%	32%	8.8%	Pneumonia, sinusitis, otitis, diarrhea
p11	CVID	5	7	2	20	F	1	90	0	20	60%	22%	41%	2%	UTI, splenomegaly, hepatomegaly, AIHA,
p12	XLA	2	7	5	16	M	1	52	0	10	89%	30%	50%	0.1%	Pneumonia, conjunctivitis, skin involvement, arteritis, diarrhea
p13	XLA	1	7	6	10	M	0	0	0	4	75%	40%	30%	0.07%	Pneumonia
p14	XLA	1	1.6	0.6	5	M	1	600	5	5	87%	46%	41%	1.1%	Guillain barre syndrome
p15	XLA	0.1	0.5	0.4	2	M	1	34	3	5	83%	63%	20%	0.4%	Pneumonia, candidiasis,
p16	XLA	5	7	2	23	M	1	700	0	64	93%	32%	54%		Otitis,
p17	XLA	0.6	9	8.6	10	M	1	200	11	31	79%	39%	38%	3.1%	Pneumonia, conjunctivitis, skin involvement, sinusitis, otitis, diarrhea,
p18	XLA	3	7.5	4.5	8	M	1	348	0.9	0.3	91%	48%	40%	0.2%	Pneumonia, otitis, diarrhea, meningitis
p19	HIGM	5	6	1	23	M	No	49	7	228	33%	50%	35%	3%	Pneumonia, conjunctivitis, sinusitis, diarrhea
p20	HIGM	5	13	8	15	F	No	586	0.7	385	62%	23%	38%	6.9%	Otitis, LAP

DOO: Date of onset; DOD: Date of diagnosis of PAD; DD: Delay diagnosis of PAD; DOD of HP infection, Cons: Consanguinity. LAP: lymphadenopathy; UTI: Urinary tract infection, IBS: Irritable bowel syndrome, AIHA: Autoimmune hemolytic anemia

The frequency of *H. pylori* infection in patients with CVID and XLA (~30%) was slightly higher than in HIgM patients (18%). Although, the rate of infection across the Iranian population is up to 80% in adults and around 50% in children using a combined

UBT and HSPA methods (19), the prevalence of *H. pylori* infection is lower in immunodeficient individuals. Repetitive and multiple administrations of antibiotics and immunoglobulins (which are therapeutic considerations for GI symptoms in PID patients) (20, 21) could be involved in low prevalence of *H. pylori* in these patients, as our PAD patients used prophylactic antibiotics including cotrimoxazole, co-amoxiclav, or clarithromycin on a daily basis. Furthermore, previous studies have also shown that the prevalence of *H. pylori* in patients with human immunodeficiency virus infection was less than that of age-matched HIV-negative controls, who also used antibiotics against opportunistic infections (22-24). Another possible explanation regarding the low rate of *H. pylori* could be receiving intravenous immunoglobulin (IVIG) containing the pooled immunoglobulin G (IgG), which contains a large amount of anti-*H.pylori* antibodies and might have a prophylactic effect.

Consistent with our results, Ardeniz O et al. found that 8 (42%) of the 23 Turkish CVID patients had *H. pylori* infection (25), while this rate is 77.2 % for Turkish population prevalence (26). Also, in other studies carried out on 25 Spanish patients and 65 Italian patients with CVID, the results indicated that 40% (27) and 41% (12) of these patients were infected with *H. pylori*, respectively. These rates were lower than Spanish (54.9%) and Italian (56.2%) normal population (26). Furthermore, Silje F. Jørgensen et al. showed that only 1 out of 53 (28) (0.018 %, significantly lower than the normal population 52%) CVID patients had *H. pylori* infection (26). On the other hand, in a study carried out on 20 Greek patients, it was reported that 70% of these patients were infected with *H. pylori* (29) which was higher than Greek population prevalence 52.1% (26). One possible explanation for the differences existing in the results of these studies could be the use of different methods to determine *H. pylori* infection in CVID patients compared with other studies. Another major reason might be the genetic variation among different studies. Moreover, since many patients with *H. pylori* infection did not present significant clinical manifestations (sometimes without any clinical manifestations), thus they were not investigated for infection with *H. pylori*. Regarding the prevalence of *H. pylori* infection in XLA and HIgM patients, Desar et al. reported one HIgM patient who was positive for *H. pylori* and five investigated XLA patients who were negative (20). In other studies, an XLA patient having *H. pylori* infection has been reported to be rare (18, 30). As mentioned earlier, low prevalence of *H. pylori* in PAD patients could be due to repetitive and multiple administrations of antibiotics and immunoglobulins (31).

Recent epidemiologic evidence revealed that *H. pylori* infection increases the risk

of gastric cancer in CVID and XLA patients (11, 12). Zullo et al. reported that the prevalence of chronic active gastritis involving both antrum and fundus was substantially higher in *H. pylori* positive (79%) than in *H. pylori* negative (20%) patients (12). In 2014, Staines Boone et al. reported a 30-year-old man infected with *H. pylori* who developed gastric adenocarcinoma in the context of XLA (11). However, our patients with *H. pylori* infection showed no signs of gastric cancer (median follow-up period of 4 years) and only one case of CVID with *H. pylori* infection had chronic active gastritis. Since the main immune response against *H. pylori* infection is mediated by regulatory and $CD8^+$ T-cells (32, 33), decreased regulatory and $CD8^+$ T-cells in CVID patients (34-36) lead to the development of chronic inflammation in those patients. Furthermore, in accordance with our results, a study reported that none of the patients with positive *H. pylori* test had atrophic gastritis, and even patients with trophic gastritis or intestinal metaplasia did not have positive *H. pylori* tests (28). As an explanation, absence of chronic *pylori* infection could be due to use of regular antibiotics and Ig replacement therapy as well as short follow-up time of our patients for experiencing the acute or chronic stage of infection.

Our results demonstrated that the serum IgA concentration was slightly lower in patients with *H. pylori* infection than in patients without *H. pylori* infection. Secretory IgA (sIgA) is the predominant Ig class in the gastrointestinal tract and plays an important role as the first line of defense against bacterial and viral antigens. IgA-deficient individuals show a higher frequency of GI infections caused by enteric pathogens such as *Giardia* and *H. pylori* (20, 37-39), a co-infection pattern which was observed in our cohort. Desar et al. reported that serum bactericidal activity in *H. pylori* infected CVID patients was considerably lower than in the seropositive control group, which can prove the role of Ig in serum bactericidal activity (31). Thus, IgA has an important role in the mucosal immunity against *H. pylori* and parasite infections.

We observed that the prevalence of diarrhea was higher in *H. pylori* positive than *H. pylori* negative patients, but this difference was not statistically significant. Intermittent or persistent diarrhea has been described as the most common GI symptom in PAD patients (40), particularly in 20–60% of CVID patients (41, 42). Since the high acidic environment in the stomach is considered as a major non-specific barrier against the entrance of enteric pathogens (43, 44), then hypochlorhydria resulting from *H. pylori* infection can induce chronic diarrhea in the patients with *H. pylori* (45). Indeed, *H. pylori* could be involved in the induction of gastric epithelial cells by cytokine-mediated like IL-8 production (46,

47). However, other mechanisms could also be responsible for an acute infectious syndrome, which should be investigated in future.

In conclusion, our study indicated that most of the PAD patients with *H. pylori* infection did not manifest any gastrointestinal disease. Meanwhile, the present study found that there was no significant association between *H. pylori* infection and development of the gastric disease in PAD patients. However, more studies with a longer follow-up and a larger number of patients should confirm our study.

Conflict of interest: The authors declare that they have no conflict of interest.

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