



A STUDY OF THE PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN PATIENTS OF CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA TREATED AT A TERTIARY LEVEL HOSPITAL IN COMPARISON WITH HEALTHY ADULTS OF NORTH INDIA

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ABSTRACT

Helicobacter pylori (*H. pylori*), a ubiquitous Gram-negative bacteria, was initially discovered as the causative organism of peptic ulcer disease/gastritis. Various studies have pointed towards an increased prevalence of *H. pylori* infection in ITP and platelet recovery following *H. pylori* eradication therapy. In our study conducted at a tertiary-level hospital in north India, 50 consecutive patients over 16 years with chronic ITP were enrolled and compared with 50 healthy adults. The prevalence of *H. pylori* infection detected by IgG anti-*H. pylori* antibodies was 38% (n=19). In comparison, in the control group, it was 46% (n=23), a non-significant association between chronic ITP and *H. pylori* infection. It was also noted that the male sex carried a significant risk for *H. pylori* infection. Low socio-economic status was an important risk factor for *H. pylori* infection. In conclusion, when matched, the seroprevalence of *H. pylori* infection in adult ITP patients is not dissimilar from that of the general population of North India.

KEYWORDS

INTRODUCTION

Primary immune thrombocytopenic purpura (ITP) is an immune-mediated acquired disease and is characterized by transient or persistent isolated thrombocytopenia ($<100 \times 10^9$). Secondary ITP is an entity which has a triggering factor, which can be autoimmune diseases or chronic infections like Hepatitis C (HCV), human immunodeficiency virus (HIV) and *Helicobacter pylori* (*H. pylori*). (1) Approximately 80% of the population may be infected with *Helicobacter pylori* (*H. pylori*) by age 20. In India, the prevalence of *H. pylori* infection is 22%, 56%, and 87% in the 0-4 years, 5-9 years, and 10-19 years age groups, respectively. (2)

Helicobacter pylori (*H. pylori*) is a ubiquitous gram-negative bacteria initially discovered in 1982 as a causative factor of peptic ulcer disease/gastritis. It has also been implicated in the pathogenesis of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. (3) Gasbarrini et al. showed a high prevalence of *H. pylori* infection in 18 patients with ITP and reported a good response in most patients with bacteria eradication. (4) Following this, numerous studies, mainly in Japanese and Spanish patients, have pointed towards an increased prevalence of *H. pylori* infection in ITP and platelet recovery following *H. pylori* eradication therapy. But conflicting results have been reported in at least 4 cohort studies in American, Spanish, Italian and French patients, where no relation between ITP and *H. pylori* was noted. (5-8)

The various proposed mechanism for explaining the development of ITP in *H. pylori* varies from molecular mimicry between the bacterial antigen (Urease B) and the platelet surface glycoprotein IIIa resulting in platelet aggregation to down-regulation of the reticuloendothelial system and induction of Th1 phenotype. Still, no proven mechanism exists regarding the possible mechanism. (9, 10). Two recent studies proposed an alternative pathway for cITP induction by *H. pylori* in which an immunocomplex forms comprising the *H. pylori* antigen Lpp20, *H. pylori*-specific antibodies, and platelets (11,12). These studies demonstrated that the *H. pylori*-specific antibodies (anti-Lpp20 antibodies) were present at significantly higher levels in responders compared to non-responders and in *H. pylori*-uninfected cITP patients. The anti-Lpp20 antibodies reacted to *H. pylori* Lpp20 but not platelets; however, Lpp20 can bind to platelets, resulting in immunocomplex formation and platelet destruction. This alternative pathway could be responsible for the clinical manifestations

mentioned above, as *H. pylori*-specific platelet-binding antigens could decrease quickly and temporally even in responders with unsuccessful eradication therapy. Lpp20 is released from *H. pylori* and may have high antigenicity (13,14).

It is in the light of these factors and the absence of any Indian data vis-à-vis *H. pylori* and chronic ITP that we planned this study. We intended to assess the prevalence of *H. pylori* infection in adult patients of chronic ITP, identify any relation between *H. pylori* infection and the clinical features of ITP patients and the association between the socio-economic status and the *H. pylori* infection. This data was compared with age and sex-matched healthy, unrelated controls for interpretation.

MATERIALS AND METHODS

Our study was conducted at a tertiary-level hospital of north India. 50 consecutive patients age >16 years with chronic ITP (thrombocytopenia lasting for more than six months) were enrolled after excluding secondary forms of ITP like HIV, HCV, SLE and APLA syndrome, chronic drug intake, CLL etc. To assess the relationship between socio-economic scale and *H. pylori* infection, note of patient's occupation, monthly household income and education was made. Based on the modified Kuppuswamy socio-economic scale, they were divided into five classes: upper, upper middle, lower middle, upper lower and lower class. (15)

All patients were asked to fill a standardized questionnaire for gastrointestinal symptoms or past medical history at the time of the testing. It included heartburn, abdominal bloating, burping, nausea, vomiting, and epigastric pain, constipation, past history of upper gastrointestinal bleed/ulcer and history of NSAID intake.

The presence of *H. pylori* infection was detected by screening the patient's serum for IgG antibodies. Serum samples were drawn from cITP patients and stored at -80°C until serological testing was done. An Enzyme-Linked Immunosorbent Assay (ELISA) method (Accubind ELISA Microwells Anti- *H. pylori* IgG, Monobind Inc, Lake Forest, CA, USA) was used to detect the presence of IgG antibodies against *H. pylori* in the patients and controls.

For control, 50 healthy age and sex-matched hospital visitors were enrolled into the study after informed consent. None of them had a

history of thrombocytopenia. Their *H. pylori* infection status and history were not known.

Statistical Analysis

Patients were compared with controls and *H. pylori* positive patients with *H. pylori* negative patients for chronic ITP characteristics. Results were expressed as mean $\pm$ standard deviation (SD) or as median (range). A chi square test was used to compare the categorical data, and the non parametric Mann-Whitney test was used to compare quantitative data. A 2-tailed *P* value of less than 0.05 was considered significant.

RESULTS

Out of the 50 patients of chronic ITP (cITP) enrolled for the study, 34 (68%) were females and 16 (32%) were males. (Table-1) The prevalence of *H. pylori* infection as detected by the presence of IgG anti-*H. pylori* antibodies was 38% (n=19), while in the control group, it was 46% (n=23). After applying the chi square test, there was a non-significant association between cITP and *H. pylori* infection (considering $p < 0.05$ as significant). (Table 2) It was also seen that male sex carried a significant risk for *H. pylori* infection. $p = 0.014$ (< 0.05), OR=4.63, RR=1.96. No such correlation was found in the control group ($p = 0.670$) (Table-3)

Patients were analysed based on the standardized questionnaire for symptoms of dyspepsia and whether they could be predict the presence of *H. pylori* in these patients. The presence of dyspeptic symptoms was not predictive of *H. pylori* infection. H/o of NSAID intake was noted in 3 *H. pylori* infected patients and in 4 non-infected group and was non-significant. (Table-4)

Details regarding the patient's education, monthly household income and occupation were noted and categorized into 5 socio-economic classes based on the modified Kuppuswamy score. No correlation was found between the socio-economic status and *H. pylori* infection in the cITP patients. (Table-5)

DISCUSSION

Several studies have reported *H. pylori* infection in patients of autoimmune diseases, particularly with idiopathic thrombocytopenic purpura (ITP). It is well known that the seroprevalence of *H. pylori* infection in healthy varies greatly from country to country. It has decreased over the last decade in industrialized nations and increased with age. Various epidemiological surveys done in India indicate that seroprevalence of *H. pylori* is 20%-50% in children under the age of five, increasing to 80%-90% by age twenty and remaining constant thereafter (16-19). A study was done by Antony et al (20) in a tertiary care centre in South India on 50 patients with ITP over 6 months. Biopsy from the antrum was subjected to a Rapid urease test to detect *H. pylori*. The prevalence of *H. pylori* infection in ITP patients was 36% and the majority of patients were male (78%). Our study had similar results as *H. pylori* was prevalent in 38% of patients and 62% of male patients had Anti *H. pylori* IgG antibodies in their serum. Our study was one step further since we used a less invasive procedure to come to a similar conclusion.

In a case study done by Mungazi et al (21), out of three hundred patients who came with complaints other than upper gastrointestinal system, the prevalence of *Helicobacter pylori* infection was found in 67.7 percent (203/300) patients. It was significantly correlated with increasing age ($p = 0.012$), sharing of a bed with siblings during childhood ($p = 0.013$) and the mode of sanitation methods ($p = 0.023$). No association was found between *H. pylori* infection and other risk factors such as; gender, level of education, employment status or number of rooms in a house. Our study was similar since the prevalence of *H. pylori* was 46% in asymptomatic controls.

In this study, 50 patients of chronic ITP were tested for IgG anti *H. pylori* antibodies by ELISA method and compared with healthy age and sex matched controls. We did not find any significant association between *H. pylori* infection and cITP. The prevalence in the cITP group was 38%, which was not statistically different from the 46% detected in control group. The slightly lower prevalence rates in both groups compared with previous rates from India may be due to the lower mean age in our study. The low prevalence of *H. pylori* as in our study was also reported by at least 4 cohorts studied, which did show no significant difference between the patients of ITP and infection (6, 7, 22, 23)

In the *H. pylori* positive group of chronic ITP patients, male sex was a significant risk factor with 10 of the 16 patients (62.5%) being positive for *H. pylori*. The male predominance of *H. pylori* infection in adults is a global and homogeneous phenomenon and has been established in many previous epidemiological studies. In a meta-analysis done by Martel et al In 18 adult populations the test of heterogeneity was not significant and male gender was significantly associated with *H. pylori* infection (summary odds ratio [OR] 1.16 [95% confidence interval (CI) 1.11, 1.22]. In 10 pediatric populations, the test of heterogeneity was of borderline significance, and the summary OR computed using a random effect model was close to 1 (summary OR 1.03 [95% CI 0.91, 1.17]). This study confirms the male predominance of *H. pylori* infection in adults as a global and homogeneous phenomenon; such predominance is not apparent in children. Differential antibiotic exposure or differential protective immunity between genders may explain the different results observed between children and adult studies.

There was no difference in the mean age between the sexes as also noted by studies by Robert Stasi et al. and Michel M et al. (6, 22, 23)

We also saw a statistically significant difference between the prevalence of *H. pylori* among various socio-economic classes. The lower socio-economic class had more patients with a positive IgG *H. pylori* antibody as compared to the upper class. A case study done by Ahmed et al, showed that the overall prevalence of *H. pylori* in India was 80% (14). *H. pylori* infection has been reported by several studies to be high in developing countries, and associated with low levels of education, low social economic status, and poor sanitation (24)

Although various studies have reported that eradicating *H. pylori* is often accompanied by a significant increase in the platelet count, little is known about the *H. pylori* associated ITP (25). Many hypotheses have been proposed about the mechanism. *H. pylori* could induce antibody in response to antigens that cross react with various platelet glycoprotein antigens. Molecular mimicry is a strongly supported hypothesis with the highly antigenic CagA antigen being responsible for the cross mimicry (26). The evidence of *H. pylori* and ITP were mostly derived from the data on *H. pylori* eradication. In a randomized controlled trial done by Suzuki et al, an improvement in platelet count was noted after *H. pylori* eradication therapy in chronic ITP patients. The titers of anti-CagA antibodies were also higher in responders as compared to non-responders (5). A meta-analysis done by Pezeshki et al. (27), a total of seven studies comprising of 228 *H. pylori*-infected patients (193 with successful eradication) were included in the analysis. The association between *H. pylori* eradication and ITP was expressed as odds ratios (OR) and 95% confidence intervals (CI). The findings showed that patients who received eradication treatment for *H. pylori* infection had significantly higher OR (OR, 8.83; 95% CI, 2.03-38.35; $P = 0.004$) than those in the non-eradicated group. Franchesi and colleagues noted the disappearance of anti-CagA antibodies following eradication therapy for *H. pylori*. (4)

Our study was a representation of the population of North India. With similar studies done in different parts of the world and India, it can thus be concluded that the diagnosis of *H. pylori* in ITP patients may be considered in the areas with high prevalence of *H. pylori*. The eradication of *H. pylori* might help improve the platelet count in ITP patients and there is a scope of improvement in the prognosis of the disease.

CONCLUSION

The finding of increased *H. pylori* infection rate in patients of low socioeconomic status corroborates with all the previous studies, stating low socio-economic status as an important risk factor for *H. pylori* infection. Dyspeptic symptoms were significantly associated with a higher *H. pylori* infection rate.

Thus, as a whole, the seroprevalence of *H. pylori* infection in adult ITP patients is not dissimilar from that of the general population of North India when matched for age, perhaps due to the early infection with the bacteria at an early age in a developing country as India.

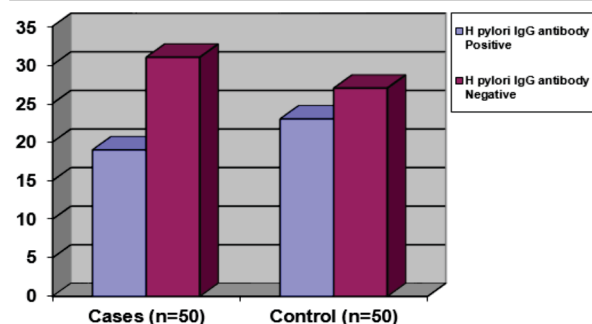
Table-1(Demographic distribution of the study subjects)

	Patients (n=50)	Controls (n=50)
AGE		
Mean	34.1	29.70
SD	13.28	8.60
Range	17-77	18-55

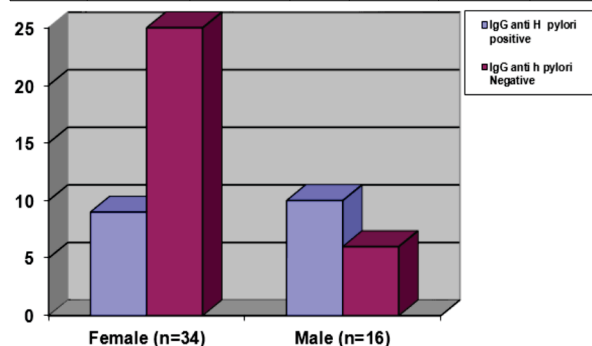
SEX	16 (32%)	18 (36%)
Male	34 (68%)	32 (62%)
Female		
PREVALENCE OF H. pylori		
Positive	19(38%)(M=10,F=9)	23(46%)(M=9,F=14)
Negative	31(62%)(M=6,F=25)	27(54%)(M=9,F=18)

Table-2(The prevalence of H pylori infection)

Anti H pylori IgG antibody	Patients (n=50)	Controls (n=50)	(p=0.41 by chi square test)
Positive	19 (38%)	23 (46%)	
Negative	31 (62%)	27 (54%)	

**Figure 1 Prevalence of H. pylori IgG Antibody in cases and controls****Table-3(Gender predilection with H pylori Infection)**

Sex	Anti H pylori IgG antibody-patients			Anti H pylori IgG antibody controls		
	Positive	Negative	p	Positive	Negative	p
Male	10 (62%)	6 (38%)	0.014	9	9	0.67
Female	9 (26.5%)	25(73.5%)		14	18	

**Figure 2 Gender distribution of IgG anti h pylori antibody in patients****Table-4(Questionnaire for symptoms of dyspepsia)**

	H pylori positive patients (%) n=19	H pylori negative patients(%)n=31	p
Heart burn	7	22	0.425
Abdominal bloating	10	21	0.285
Nausea	3	8	0.407
Vomiting	0	3	0.162
Epigastric pain	6	12	0.610
Constipation	8	11	0.680
h/o GI bleed	1	4	0.382
h/o NSAID use	3	4	0.775

Table-5(socioeconomic status and h pylori infection)

Socioeconomic Status scale	IgG H pylori antibody		p
	Negative n=31	Positive n=19	
Upper	3	1	0.043
Upper middle	14	12	
Lower middle	12	6	
Upper lower	2	10	
Lower	0	0	

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