

## *Helicobacter pylori* Eradication in Adult Patients with Acute Idiopathic Thrombocytopenic Purpura (ITP)

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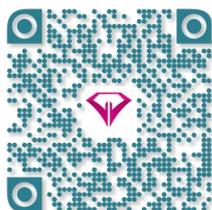
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### ABSTRACT

**Background & Objective:** Different studies have investigated the link between *Helicobacter pylori* (*H. pylori*) and extra digestive diseases such as idiopathic thrombocytopenic purpura (ITP). However, the relationship between ITP and *H. pylori* is less clear. Most of the studies in ITP have focused on *H. pylori* eradication in chronic ITP, therefore, in this study we focused on the effect of *H. pylori* eradication on chronicity of ITP in the adult patients with acute ITP.

**Materials & Methods:** Eighty five patients with acute ITP whose platelet counts were less than  $30 \times 10^9/L$  entered into the study. Urea breath test (UBT) was carried out for all the patients and based on the results they were divided into 3 groups: Group I: *H. pylori*-positive patients who underwent standard triple therapy. Evaluation of *H. pylori* eradication for this group was carried out in one and six months after the treatment. Group II: ITP patients negative for *H. pylori* and Group III: ITP patients positive for *H. pylori* but without eradication therapy.

**Results:** 52 (61.2%) patients were female and 33 (38.8%) were male with the mean age of  $34.8 \pm 12.2$  years old. There were no significant differences between either the mean age and gender or the mean platelet count at the baseline among the groups, but there was a statistical significant difference in the mean platelet between the groups at the end of the first month. However, significant difference was not seen in the mean platelet count in months 2 to 6. The chronicity in group I was significantly less than group III. Also, the chronicity rate in non-infected ITP patients was lower than the *H. pylori*-positive patients ( $P=0.03$ ). Likewise, the chronicity rate was lower in the intervention group than in the control group ( $P=0.035$ ).

**Conclusion:** Our results showed that eradication of *H. pylori* can reduce the chronicity rate in adult patients with ITP. Further studies on larger number of patients with longer follow-up are recommended.

**Keywords:** Chronicity, *Helicobacter pylori*, Idiopathic Thrombocytopenic Purpura, Platelet, Urea breath test



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### Introduction

The term idiopathic thrombocytopenic purpura (ITP) indicates thrombocytopenia without any external etiologic factors, ruling out other diseases associated with secondary thrombocytopenia. ITP is divided into two types; acute and chronic (1). Thrombocytopenia for less than 6 months is indicated as acute ITP. Acute ITP is rare in adults, but over 90%

is converted into chronic form which requires prolonged treatment (2,3).

*Helicobacter pylori* (*H. pylori*) is a spiral gram-negative bacteria that colonizes in the mucosal layer of the stomach. It is usually associated with a variety of gastrointestinal disorders including gastric mucosal atrophy, chronic gastritis, peptic ulcer and gastric adenocarcinoma (4-6).

The exact mechanism of *H. pylori*-associated ITP is still uncertain, but some studies have suggested that cytotoxin-associated gene A (Cag A) stimulates the development of anti-CagA antibodies (Abs). These antibodies may cross-react with the platelet surface antigens resulting in ITP (7).

*Helicobacter pylori* prevalence in patients with ITP is the same as in other people. Its prevalence is approximately 30% in the developed countries and more than 80% in developing countries in adult population. Several studies have shown the relationship between *H. pylori* and gastric diseases, including autoimmune diseases (8,9), but this type of relationship is not clear about ITP. For example, elimination of *H. pylori* in the patients with ITP in Japan led to the improvement in disease, but this finding was not confirmed in America. Thus, it is not reasonable to stick to routine testing of *H. pylori* in the patients with ITP. However, some references recommend *H. pylori* testing in adults but it is not applicable in the children with ITP (2). The acute type of ITP in 28% of children is converted to chronic types which need treatment (1). Acute ITP is rare in adults, but over 90% is converted into chronic form and require lengthy treatments (2,3). In new references, chronic ITP is the condition with platelet counts lower than  $50 \times 10^9/L$  for 3 months and longer as well as lack of response to splenectomy (2). Despite various therapies, ITP in adults is a serious disease compared to children and has a mortality rate of 5% (mostly due to an intracranial hemorrhage).

Several studies have investigated on *H. pylori* eradication in ITP and reported that *H. pylori* eradication can lead to an increase in platelet counts (10,11). However, other studies were unable to confirm the beneficial effect of bacterial eradication in ITP (12,13).

In a systematic review by Arnold *et al.*, eleven studies were reviewed. It was concluded that *H. pylori* treatment for ITP patients without *H. pylori* infection is of little benefit in comparison with *H. pylori*-positive ITP patients. These data showed that there may be a causal link between *H. pylori* infection and ITP in some patients (14).

In another study, it was concluded that treatment of *H. pylori* in ITP patients is indicated only in the patients with *H. pylori* positive test (15). Shaikh *et al.*, in their study showed an association between *H. pylori* and chronic ITP and recommended the eradication of infection in positive cases at diagnosis (16).

In a systematic review conducted by Stasi *et al.*, twenty surveys were appraised and it was revealed that response rates in countries with a higher prevalence of *H. pylori* infection in the patients with milder degrees of thrombocytopenia were higher. As a result, screening for *H. pylori* in Japan and countries with a high prevalence is valuable but for countries

like America where the prevalence of *H. pylori* infection and the response rate is low, the cost-benefit ratio should be assessed (17). Semple *et al.*, demonstrated that in the presence of antibodies against platelets, gram-negative bacteria lipopolysaccharide can markedly affect Fc-mediated platelet phagocytosis (18).

Therefore, due to controversial outcome of *H. Pylori* eradication in ITP, the present study was designed to elucidate the role of *H. pylori* eradication as a preventing factor of ITP chronicity.

## Materials and Methods

This investigation was designed as a quasi-experimental study. The cases were collected among all admitted patients with acute ITP and platelet counts less than  $30 \times 10^9/L$  during the sampling period in some general Hospitals in Iran. The following variables were considered: age, gender, *H. Pylori* treatment, platelet count and chronic ITP. Criteria for the diagnosis of acute ITP included: 1) Having platelet count less than  $30 \times 10^9/L$  with normal WBC, Hb and ESR, 2) Age of eighteen years and older of both sexes, 3) Having bone marrow aspiration/biopsy (BMA/B) compatible with ITP (if it was required), 4) Ruling out pseudo-thrombocytopenia, hereditary thrombocytopenia as well as secondary ITP due to human immunodeficiency virus (HIV) infection, autoimmune disorders and drug-induced thrombocytopenia, 5) No history of previous ITP, 6) No history of splenectomy.

From eighty seven cases of acute ITP who entered in this study, two patients were excluded due to the diagnosis of HIV and myelodysplastic syndrome (MDS) and eighty five patients with acute ITP and platelet counts below  $30 \times 10^9/L$  were finally enrolled. Because of the low incidence of acute ITP in adults the number of patients was less than anticipated. After identifying patients with acute ITP eligible to participate in the study and also after disease stability regardless of response to treatment, the cases were underwent UBT if they had no history of drug taking to interact with the mentioned test according to their medical histories. In the case of recent drug consumption, UBT was performed after one month of drug discontinuation. Patients were divided into 3 groups based on the UBT results: Group I) eradication of *H. pylori* in infected ITP cases using standard triple therapy by Amoxicillin 1000 mg twice daily, and Clarithromycin 500 mg twice daily and Pantoprazole 40 mg twice daily for 7 days, Group II) *H. pylori* uninfected cases with standard treatment of ITP) and Group III) patients infected with *H. pylori* which did not receive eradication therapy. All patients received standard therapy included corticosteroid with or without intravenous immunoglobulin (IVIG). UBT was carried out at the first and the sixth month after the onset of therapy.

**Table 1. Comparisons of mean platelet counts in 3 groups during the study (mean±SD)**

Time of platelet counting	Group I Plt no /uL	Group II Plt no /uL	Group III Plt no /uL	P-value
At the beginning of study	7161±5222	9000±5649	8866±7233	0.34
At the end of the 1 <sup>st</sup> month	59258±43570	107958±88983	50233±34708	0.001
At the end of the 2 <sup>nd</sup> month	96903±46286	112916±77229	100933±67747	0.64
At the end of the 3 <sup>rd</sup> month	128580±54800	139958±64889	121933±62709	0.55
At the end of the 4 <sup>th</sup> month	151645±65400	148666±69850	138226±70035	0.73
At the end of the 5 <sup>th</sup> month	157225±61809	153875±77440	147733±69209	0.86
At the end of the 6 <sup>th</sup> month	170741±55390	162916±71482	157400±79446	0.75

In the first group, the patients whose *H. pylori* infection were not eradicated (UBT was positive in the first month) or the patients who had recurrent infection (UBT was positive at the end of the six<sup>th</sup> month) were transferred from group I to group III and data analysis was done with and without them. These groups received standard treatment for ITP. The platelet count was measured every month up to six months after the start of the treatment. All data required for this study included age, sex, time of entry into the study, tests requested, and date of treatment which were entered into the questionnaires.

Inclusion criteria were as follows: 1) Patients over eighteen years of age, 2) Patients who have not been elapsed from disease onset more than six months (acute ITP), 3) Other causes of thrombocytopenia were excluded. Exclusion criteria for the study were as follows: 1) A serious illness such as heart, liver and kidney diseases, and neoplastic disorders, 2) History of previous treatment for *H. pylori*, 3) Allergic reaction to therapies used for *H. pylori*, 4) Distinct gastrointestinal signs and symptoms for the pharmacologic intervention as well as treatment for *H. pylori*, 5) Secondary causes of ITP.

Chi-square (for qualitative variables), independent t-test (in the case of normally distributed quantitative variables) and Mann–Whitney U test (in the case of non-normal distribution of quantitative variables) were used for the statistical analysis.

In view of medical ethics, there was no compulsion to participate in the study. All patients were initially explained that the information was completely confidential and used solely for the scientific goals. Also, unknown effect of *H. pylori* treatment on patients with ITP was explained to the patients and a written informed consent was obtained from all the participants.

Ethically, there was no need to treat all patients with positive UBT, because all the patients with *H. pylori* did not have indications for treatment. Additionally, cases with indication for *H. pylori* treatment (like peptic ulcer histories) were excluded.

## Results

UBT was positive in sixty one patients (71.8%). Thirty seven of them (60.6%) were female and twenty four (39.4%) were male. UBT in twenty four patients (28.2%) was negative; among them fifteen (62.5%) were female and nine (37.5%) were male.

Thirty six patients (UBT positive group I cases) underwent eradication of infection. Twenty four patients in the second group (UBT negative cases) and twenty five patients in group III (UBT positive cases without eradication of infection) were enrolled. Three cases of group I and two cases of group II were excluded due to lack of *H. Pylori* eradication and infection recurrence at the end of the 6<sup>th</sup> month, respectively. These cases were placed in group III and analysis was carried out with and without them. No significant difference was observed between gender and age of the patients in 3 groups.

The mean platelet count in different groups was not statistically significant at the beginning of the study ( $P=0.34$ , [Table 1](#)). At the end of the first month, mean differences of platelet counts between 3 groups of patients was statistically significant ( $P=0.001$ , [Table 1](#)). There was a significant difference between platelet mean in groups I and II ( $P=0.01$ ) as well as groups II and III ( $P=0.002$ ) at the end of the first month, but there was no significant difference between groups I and III ( $P=0.83$ ). There were no significant statistical differences between mean platelet count in 3 groups after the end of the second month to six months

( $P=0.64$ ,  $0.55$ ,  $0.73$ ,  $0.86$  and  $0.75$ , respectively). It is noteworthy that the mean platelet count difference between the groups excluding 5 transmitted patients from group I to group III, showed no statistically significant difference during the second to sixth months either.

Platelet count higher than  $100 \times 10^9/L$  was considered as a cutoff point for the assessment of chronicity at the end of the sixth month. There was no significant difference between groups I and II in terms of chronicity ( $P=0.22$ ). However, chronicity of the disease in group I was statistically significant and much lower than group III ( $P=0.018$ ). The disease chronicity in group I without excluded 5 transferred cases (3 refractory patients and 2 patients with relapsed *H. pylori* infection) was significantly lower

than group III ( $P=0.016$ ) at the end of the sixth month. The chronicity rate in the patients without *H. pylori* infection (Total Groups I and II) was significantly lower than in the patients with *H. pylori* infection (group III), ( $P=0.03$ ). The mean platelet count elevation in groups I, II and III between the baseline and the end of the sixth month showed no significant difference ( $P=0.64$ ). No significant difference was also observed between treatment groups at the end of the sixth month ( $P=0.67$ , [Table 2](#)).

As it is shown in [Table 3](#), the likelihood of having platelet count higher than  $100 \times 10^9/L$  was 0.14 in group III compared to group I ( $P=0.02$ ) which showed significant effect of *H. Pylori* eradication on ITP chronicity despite age, gender, splenectomy and baseline platelet count.

**Table 2. Comparison of different treatment procedures performed during the study**

Procedure	Group I	Group II	Group III	Total
Corticosteroid therapy	25 (80.6%)	20 (83.3%)	21 (70%)	66 (77.6%)
Splenectomy	4 (12.9%)	2 (8.3%)	4 (13.3%)	10 (11.8%)
Others*	2 (6.5%)	2 (8.3%)	5 (16.7%)	9 (10.6%)
Total	31 (100%)	24 (100%)	30 (100%)	85 (100%)

$P=0.67$

\*Others: Rituximab, Cellcept, Cyclophosphamide, Cyclosporine, Azathioprine

**Table 3. The logistic regression model results of *H. Pylori* eradication on ITP chronicity in association with and without age, gender, splenectomy and baseline platelet count**

Variable	Odds ratio	(95%) CI	P-value
<i>H. Pylori</i> eradication	Group I (with <i>H. Pylori</i> eradication)	1.00	-----
	Group III (without <i>H. Pylori</i> eradication)	0.14	0.03-0.75
Age	1.003	0.942-1.068	0.922
Gender	Female	1.00	-----
	Male	0.95	0.22-4.04
Baseline platelet count	1.00	1.00	0.22
Splenectomy	No	1.00	-----
	Yes	1.99	0.2-19.8

## Discussion

A link between ITP and *H. pylori* infection was proposed by Gasbarrini *et al.*, who showed a significant increase in platelet count after *H. pylori* eradication in ITP patients infected with these bacteria (19).

Eradication of *H. pylori* infection has been variably associated with a platelet response in the patients with ITP. The response occurs in some ITP patients infected with this bacterium but not in all patients. Eradication

therapy seems to be more effective in the patients with less severe ITP (platelet count  $>30 \times 10^9/L$ ) and a shorter duration of disease. Most of the studies regarding *H. pylori* infection and ITP have been carried out on the patients with chronic ITP, but patients with chronic ITP have already experienced various treatments that have affected their platelet count. Therefore, the present survey on acute ITP in adults is one of the few existing studies. According to our results, no significant difference was observed for ITP chronicity at the end of the six<sup>th</sup> month between the groups with and without *H. pylori* infection. The first report about ITP patients whom *H. pylori* infection were eradicated was provided by Gasbarrini *et al.*, (19) and some studies were conducted by other investigators (20-23). Gan *et al.*, found that the overall platelet response rate was 30% which was similar to those reported in America but was lower compared to the studies done in Japan and Europe. They also showed that there was no platelet recovery in the patients who demonstrated initial response (24). Baseline platelet count over  $30 \times 10^9/L$  and previous treatments for ITP, such as prednisone, are factors that affect the response to eradication of *H. pylori* infection. In our study, the patients had no history of treatment with corticosteroids. However, our study differs from other studies due to the inclusion of patients with a platelet count less than  $30 \times 10^9/L$ . Veneri *et al.*, showed that there was no significant benefit of *H. pylori* infection eradication in the patients with marked thrombocytopenia, however, they showed that early eradication therapy was more effective in their patients as the thrombocytopenia was moderate (25). Our study suggests that *H. pylori* infection may be a cause of ITP chronicity which is hoped to be controlled by eradicating infection. The mechanism by which *H. pylori* may cause ITP has not been established, but a chronic immunological stimulation or immunological mimicry between *H. pylori* antigens and platelet has been proposed as a cause of ITP (16). The protein Cag-A (cytotoxin associate gene-A) is one of the molecules which has molecular cross-reaction with platelets. This protein is more prevalent in Asian countries (such as Iran, Korea and Japan) than in Western countries (18). As a result of *H. pylori* eradication, Cag-A antibody is disappeared and the platelet count is increased in ITP patients. Difference in *H. pylori* antigen genotype (prevalence of *H. pylori* with positive Cag-A) may be increased in the patients with ITP and platelet which influence the treatment outcome. Although, we have not tested for the presence or absence of this protein in our patients, the significant differences seen in ITP chronicity in our groups may be due to the differences

in *H. pylori* Cag-A protein. In most studies, cases with a slight decrease in platelet count have shown a better response to treatment (4). Suzuki *et al.*, found that the eradication of *H. pylori* infection in the patients with ITP was associated with a platelet response of 46.2% in the eradication group and 0% in non-eradication group. They also showed that the platelet response was significantly more common in the patients with infection sustained by CagA-positive strains of *H. pylori* (22).

Prevalence of ITP at older age in *H. pylori*-positive patients compared to *H. pylori*-negative patients may be related to the long-term underlying susceptibility to *H. pylori* infection (3,22). However, in our study there was no significant difference between the groups in terms of age. The results of our study indicated that eradication of *H. pylori* infection in the patients with acute ITP will prevent the chronicity of the disease because the platelet count higher than  $30 \times 10^9/L$  in groups I and II (which were negative for *H. pylori* infection at the end of the six<sup>th</sup> month) was more common than in group III (*H. pylori* infection at the end of the six<sup>th</sup> month). These results showed an association between *H. pylori* infection and ITP, thus, eradication of *H. pylori* can diminish the chronicity of ITP. At least in part, these findings suggest that screening of the patients with acute ITP in order to eradicate *H. pylori* infection can prevent ITP chronicity in a number of patients with *H. pylori* infection. Finally, we should mention that the results of the present study cannot be compared with the studies that have been conducted on the patients with chronic ITP infected with *H. pylori*, because the patients with chronic ITP have already a milder or moderate thrombocytopenia due to use of corticosteroids. Rather, patients with acute ITP and severe thrombocytopenia were entered in our study and received corticosteroids afterwards.

## Conclusion

Our study confirm some of the previous results and suggest that *H. pylori* infection may be a cause of ITP. Therefore, eradication of *H. pylori* infection in patients with acute ITP may prevent the chronicity of ITP. However, further studies on larger number of infected and non-infected patients with acute ITP are recommended to confirm these results.

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contributed to the design of research study and also drafting the manuscript. Dr Mazloomzadeh analyzed the data and Dr Razavi-Dizaji, Miss Ghadimi and Miss Baba Ali contributed to the reagents, Lab works and experiments. This work was fully supported by the grant from Zanjan University of Medical Sciences, Zanjan, Iran.

## Conflict of Interest

Authors declared no conflict of interest.

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