Original article

H.Pylori Associated with Immune Thrombocytopenic Purpura.

Baan A. Mtashar BSc . Microbiology (corresponding author)*
Zeyad A. Shabeeb. (Ph.D Immunology)* **Zainab F.Ashoor. (Ph.D Immunology)****

Abstract:
Background: Idiopathic thrombocytopenic purpura (ITP) is a hematological disorder characterized by sensitization of platelets by autoantibodies leading to platelet destruction.). Recently, H. pylori has been found to be associated with ITP and its eradication has shown improvement in platelet count
Objective: To determine the association of *Helicobacter pylori* infection in patients presenting with idiopathic thrombocytopenic purpura (ITP).
Materials and Methods: From October 2012 to March 2013, fifty adult patients with ITP and fifty age and sex matched healthy controls were investigated for the presence of *H. pylori* infection by *Helicobacter pylori* detection IgG and IgM antibody using ELISA method.
Results: *H. pylori* IgM and *H. pylori* IgG: twelve patient (22%) and thirty nine patients (78%), of ITP patients had a positive expression *H. pylori* IgM and *H. pylori* IgG, respectively compared with three (6%) and thirty one (62%) patient in the control group**.** There was statistically significant difference between the mean of ITP patients and healthy control groups.
Conclusion: The study confirms the existence of an association between *H. pylori* infection and ITP.
Keywords: H.pylori, ITP

Introduction:

Idiopathic thrombocytopenic purpura (ITP) is a hematological disorder characterized by sensitization of platelets by autoantibodies leading to platelet destruction. Although its cause remains unclear, ITP is associated with several diseases, including infections [1].

Helicobacter pylori is a Gram-negative microaerophilic bacterium that colonizes the human stomach of more than 50% of the world population. It is recognized as the causative agent of active chronic gastritis and is the predominant cause of peptic ulceration, i.e., gastric and duodenal ulcers [2]. Additionally, *H pylori* is a cofactor in the development of both adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphomas, and therefore has been designated as a class I carcinogen by the World Health Organization [3]. Recently, H. pylori has been found to be associated with ITP and its eradication has shown improvement in platelet count [4].

Many hypotheses have been advanced about the mechanisms by which *H pylori* may cause ITP [3]. One of them is molecular mimicry; according to which *H pylori* could induce antibody production in response to antigens that cross react against various platelet glycoprotein antigens [5]. The possible role of CagA-positive strains as a pathogenic candidate for ITP was recognized in two molecular studies. The first showed a decline in platelet-associated immunoglobulin G in ITP patients after the eradication of *H. pylori* infection as well as the existence of a molecular mimicry between those antibodies and the CagA protein. The second study demonstrated that CagA antibodies cross-react with a peptide specifically expressed by platelets of patients with ITP.This study, as well as supporting an association between CagA and ITP, also proposed a possible explanation for the fact that ITP may occur in only a small subset of patients infected by CagA-positive strains [3].

The recent data of an Italian group, showing that the prevalence of the *H pylori* Cag A gene was significantly higher in patients with ITP than in a control group [6]. Other putative targets of molecular mimicry are Lewis (Le) antigens, which are expressed by *H pylori* in a strain-specific manner. Le antigens adsorb to platelets and might serve as targets for anti-Le antibodies in patients with an appropriate genetic background[7], another hypothesis suggests that molecular mimicry of *CagA* or Lewis antigens and platelet antigens may initiate the development of ITP, but with time continued platelet destruction and epitope spreading may lead to the development of chronic thrombocytopenia refractory to eradication of *H pylori* infection [8] .This model is reminiscent of the role played by *H pylori* in the development of MALT lymphomas, which initially may respond to bacterium eradication but may subsequently develop new mutations leading to autonomous disease [9].

Recently, Semple and colleagues demonstrated that in the presence of antiplatelet antibodies, the LPS of Gram negative bacteria can significantly enhance Fc-dependent platelet phagocytosis [10, 11]. These results suggest that infectious agents in combination with antiplatelet antibodies could affect platelet destruction *in vivo*, which may be at least one explanation for why thrombocytopenia worsens in some patients with ITP during infections and, alternatively, resolves in other patients with ITP who are treated with bacterial eradication therapy [12].

Other studies have shown that some strains of *H pylori* bind von Willebrand factor (VWF) and induce glycoprotein Ib (GPIb) – and FcRIIa-dependent platelet aggregation in the presence of *H pylori* antibodies [13]. Activation may promote platelet clearance and antigen presentation, which augments production of antibacterial antibodies. Somatic mutation may lead to the development of antibodies that either recognizes bacterially derived factors that

bind to platelets or cross-react with platelet antigens [14].

Both *H pylori* infection and ITP are associated with a polarized Th1-type phenotype [15, 16]. Accordingly, it may be speculated that *H pylori* infection creates an immunological environment that facilitates the onset and/or persistence of ITP [17].

They document induction of platelet aggregation by *H pylori in vitro* and show that this effect is strain-dependent. Using both the proaggregatory strain (Hp49503) and non aggregatory strains (Hp42504, Hp51932), they demonstrate an essential role for P-selectin and Hp IgG antibody (Hp IGs) in *H pylori*–induced platelet aggregation. This reaction was completely inhibited by anti –P-selectin antibodies. The presence of *H pylori* was shown by demonstration of Hp-specific urease gene fragment in the aggregates. They propose that binding of bacteria/Hp IGs to platelet FcγRIIA receptor activates platelets to release granules and to induce surface P-selectin and von Willebrand factor, leading to aggregation. They also looked into platelet apoptosis evidenced by annexin V binding and membrane blebbing, and observed that both proaggregatory and non aggregatory strains induced apoptosis. Taken together, they conclude that platelet aggregation and apoptosis induced by certain strains of *H pylori* leads to thrombocytopenia [18].

Materials and methods:

This study included 100 subjects, 50 of them were apparently healthy volunteers who considered as a control group, while 50 serum samples were collected from ITP patients. The sampling and work conducted at National Center of Hematology / Al-Mustansiriya University from October /2012 to March /2013.

The average age of ITP patients was 17 – 69 years and gender were 14 males and 36 females, which matched to controls group.

All patients with ITP, defined according to the criteria set forth in the American Society of Hematology (ASH) Guidelines [19].

Complete blood count and examination of peripheral blood smear were done to confirm the diagnosis of ITP.

H. Pylori infection was detected *H.pylori* (IgG & IgM) antibody in patients and controls sera using serological method the enzyme-linked immunosorbent assay (ELISA) (Novotech/germany) for detecting IgG and Monbind /USA) for detecting IgM.

Detection H.pylori IgG antibody.

Reagent and sample preparation:

All reagents are brought in room temperature (15 – 25˚C) before use.

Wash solution is diluted (1 + 20) with fresh deionized water.

Stability up to 60 days at (15 – 25˚C)

The patient's sera were diluted by mixing with vortex 10 µ sera +1000µ dilution buffer and incubated at least 5 min.

Assay procedure:

Dispend 100µ of standards, and diluted samples were added to the appropriate wells, mixed carefully. Covered with foil and incubated 1hour at 37˚C.After incubation the microtiter plate was washed 3 times by adding 300µ washing buffer to each wells.100µ of *H.pylori* anti- IgG conjugate was added to each well. Covered with foil and incubated 30 min at 17 - 25˚C. After incubation the microtiter plate was washed 3 times by adding 300µ washing buffer to each wells. 100µ of substrate reagent was added to each well. Covered and incubated in dark 15 min at room temperature. 100µ of stop solution was added to each well. Mixed carefully. The optical density (O.D) was read at 450 nm as the primary wavelength and 620 nm as the reference wavelength using a microtiter reader.

Calculation of the result:

The standard curve was generated by plotting the O.D (450) obtained for each of the standard concentration on (linear/linear) graph paper in a system of coordinates against their corresponding concentration (0,15,75,and 150 NTU/ml) and draw a standard calibration curve (absorbance value on the vertical y – axis , concentration on the horizontal x – axis).

Read result from this standard curve employing the absorbance value of each patient specimen Finger (1).

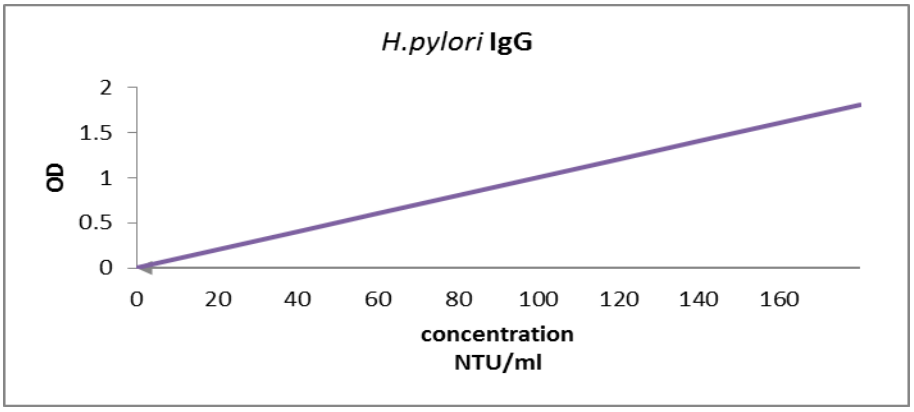

Figure 1: *H.pylori* IgG calibration curve

Detection H.pylori IgM:

Reagent preparation:

All reagents should be allowed to reach room temperature (25 ˚C) before used. Diluted 1 volume of wash buffer (20X) with 19 volume of distilled water.

The patient's sera, negative control, positive control, and calibrator were diluted by adding 10 µ+ 400µ dilution buffer mixed well with vortex and incubated diluted sample at least 5 min .

Assay procedure:

Hundred µl of diluted samples, calibrator, and controls were added to the appropriate wells, mixed carefully. Covered and incubated 30 min. at room temperature. After incubation the microtiter plate was washed 4 times by adding 300µl washing buffer to each wells and one time with distilled water. 100µl of *Helicobacter pylori* anti- IgM conjugate was added to each well. Covered and incubated 30 min at room temperature. After incubation the microtiter plate was washed 4 times by adding 300µl washing buffer to each wells and one time with distilled water. 100µl of substrate reagent was added to each well. Covered and incubated in dark 20 min at room temperature. 100µ of stop solution was added to each well. Mixed carefully. The optical density (O.D) was read at 450 nm as the primary wavelength and 620 nm as the reference wavelength using a microtiter reader.

Calculation of result

Calculate the *H. pylori* IgM EIA Index of each determination by dividing the mean values of each sample by calibrator means value.

Results

The comparison of these two categories (the ITP group fifty patients mean age (40±2.1 years) with mean Platelet count (79.1±10.1) x 10^9/L with the control group fifty healthy control with a mean age (40.2 ± 2.0years) and mean platelet count (253±72) x10^9/L showed substantial variations in the expression of *H. pylori* IgM and *H. pylori* IgG : twelve patient (22%) with mean platelet count (69.17±14.7) x10^9/L and thirty nine patients (78%) with mean platelet count (73.92 ±9. 18) x10^9/L, ITP patients had a positive expression *H. pylori* IgM and *H. pylori* IgG, respectively compared with three (6%) and thirty one (62%) patient in the control group**.** There was statistically significant difference (p=0. 02) between the mean of ITP Patients and healthy control groups In case of detection acute infection of H. *pylori* IgM Table (1).

Table (1): Comparison of acute infection *H.pylori* IgM between ITP patient and healthy control.

Parameter	**ITP Patient n=50**		**Control n=50**	**p-value**
***H. pylori* IgM**	95% Confidence interval (%)	0.84 – 0.95	0.73 – 0.86	0.02*
	Mean± SE	0.89 ± 0.027	0.79 ± 0.03	
* (P<0.05), ** (P<0.01), NS: Non-significant.				

In case of *H. pylori* IgG, there was statistically highly significant difference ($p < 0.001$) between ITP group and healthy control group Table (2) .

Table (2): Comparison of infection *H.pylori* IgG infection between ITP patient and healthy control.

Parameter	ITP Patient n=50		Control n=50	p-value
***H. pylori* IgG**	95% Confidence interval (%)	51.4 – 76.1	40.1 – 67.5	<0.001**
	Mean± SE	63.7 ± 6.14	53.8 ± 6.8	
* (P<0.05), ** (P<0.01), NS: Non-significant.				

Discussion:

The detection method in these studies was serological method detection antibodies IgG and IgM in ITP patient and control.

The prevalence of *H.pylori* – IgG positivity in the ITP group was 78% was highly significant when compared with control group 62% (P <0.001), while the prevalence of *H. pylori* - IgM positivity in the ITP group was 22% was significant when compared with control group 6%(P=0.02) .

The current result was supported by reports described by other investigators [4, 20] which found *H. pylori* infection should be considered in the differential diagnosis of all cases of thrombocytopenia, and should be eradicated in all H. pylori-positive patients with thrombocytopenia.

The actual pathogenetic mechanisms between *H. pylori* and ITP have not been clarified yet. There are many speculations about pathogenesis of ITP in patients with *H. pylori* infection. It is suggested that active *H. pylori* infection triggers humeral and cellular immune responses, which probably induce *H. pylori*-associated diseases including ITP through molecular mimicry [21]. Furthermore, many data indicate that the effect of eradication treatment for *H. pylori* probably depends on genetic factors of the host, strains of the bacterium or existing ethnic factors. According to these data, *H. pylori* infection influences the pathophysiology of ITP through various mechanisms [22, 23].

The Cag A antigen of *H.pylori* could be responsible for cross – mimicry between *H. pylori* and platelets glycoproteins[3].This hypothesis was not confirmed by Michel and his colleges who tested platelet elutes from three *H. pylori* positive patients with ITP for *H. pylori* antibodies, but none was found to be positive[24]. Conversely Takashashi and his colleagues showed that eluted platelets- associated immunoglobulin G from 12 out of 18 ITP patients' recognized *H. pylori* Cag A protein and that crass reactive antibody level decreased following *H. pylori* eradication in patient who shoed complete platelet recovery [25].

Another factor responsible for molecular mimicry may be the babA gene (blood group antigen – binding adhesion gene) expressed by some *H.pylori* strains, which codifies for antigenic epitopes which recognize sequences of Lewis blood group (Le antigens): these antigens are adsorbed by platelets and could become the target for anti – Le antibodies produced by some patient with susceptible background [7].

The development of thrombocytopenia in *H.pylori* patient infection may also depending on genetic influence .In fact, observed a difference in HLA class II allele patterns ITP patient with or without *H. pylori* infection [26].

Overproduction of TNF-α can lead to autoimmune disease. Hence, *H. pylori*–induced alterations in cytokine profiles might promote development of immune thrombocytopenia [20].

Conclusion:

The study confirms the existence of an association between *H. pylori* infection and idiopathic thrombocytopenic purpura. Further in vestigations on a larger number of patients might allow a better definition of the true prevalence of *H. pylori* infection in patients with ITP.

References:

1- Shaikh KH, Ahmed S., Ayub M. and Anwar J. Association of Helicobacter Pylori infection with Idiopathic Thrombocytopenic 2009
2- Suerbaum S, Michetti P.*Helicobacter pylori* infection. N Engl J Med.2002;347:1175-1186.
3- Stasi R. and Provan D. Helicobacter pylori and Chronic ITP.*Hematology 2008*:206 – 211
4- Rostami N, Keshtkar-Jahromi M, Rahnavardi M, Esfahani FS. Effect of eradication of Helicobacter pylori on platelet recovery in patients with chronic idiopathic thrombocytopenic purpura: controlled trial. Am J Hematol 2008; 83: 376-81
5- Fahmy HM, Twfik NM , Hashem AM and Gaafar M. Eradication Therapy for Helicobacter Pylori in Idiopathic thrombocytopenic Purpra. *Journal of American Science* 2012.8(10):117-123
6- Emilia G, Luppi M, Zucchini P, *et al*. Helicobacter pylori infection and chronic immune thrombocytopenic purpura: long-term results of bacterium eradication and association with bacterium virulence profiles. *Blood 2007*.110:3833-3841
7- Gerhard M., Rad R., Prinz C. and Naumann M. : Pathogenesis of *Helicobacter pylori* infection. *Helicobacter*.2002: 7 (Suppl 1):17-23
8- Cines DB. ITP: time to "bug off"? *Blood 2007*. 110:3818- 3819.

9- Papagiannakisa P., Michalopoulosb C., Papalexic F, Dalampourad D.and Diamantidisd DD. The role of *Helicobacter pylori* infection in hematological disorders. *European Journal of Internal Medicine*.2013

10- Yeh J., Tsai S., Wu DC, Wu JY., Liu T.and Chen A. P-selectin-dependent platelet aggregation and apoptosis may explain the decrease in platelet count during *Helicobacter pylori* infection. *Blood 2010*. 15 (21g): 4247-4253

11- Akl H.K., Mohamed H.E. and El-Hady H.A. Usefulness of *Helicobacter Pylori* Eradication for Platelet Recovery in Egyptian Idiopathic Thrombocytopenic Purpura Patients. *Life Science Journal.2012:* 9(1) :826- 829

12- Semple JW., Aslam R., Kim M, Speck ER., Freedman J.. Platelet-bound lipopolysaccharide enhances Fc receptor mediated phagocytosis of IgG-opsonized platelets. *Blood 2007*. 109:4803-4805.

13- Byrne MF., Kerrigan SW., Corcoran PA., *et al*. *Helicobacter pylori* binds von Willebrand factor and interacts with GP Ib to induce platelet aggregation. *Gastroenterology*. 2003:124:1846-1854

14- Cines Campuzano-Maya G. Proof of an association between Helicobacter pylori and idiopathic thrombocytopenic purpura in Latin America. *Helicobacter*.2003:12 (3): 265-273

15- Stasi R. .*Helicobacter pylori* and ITP where Do we stand, European Hematology2007 : 62-64.

16- Guo C, Chu X, Shi Y, *et al*. Correction of Th1-dominant cytokine profiles by high-dose dexamethasone in patients with chronic idiopathic thrombocytopenic purpura. *J Clin Immunol*.2007: 27:557-562.

17- McCrae KA. Helicobacter pylori and ITP: many questions, few answers. *Blood*.2004. 103:751-752.

18- Ahn YS.(2010),Triple play of H.Pylori in ITP.Blood ;115:4155- 4156

19- George JN, Woolf SH, Raskob GE, et al. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. Blood. 1996;88: 3-40.

20- Zahran S.M., Hamed N.A., Dafrawy M, AbdelazizH.K. and Morsi M.G.Study of host immunity in patients with *Helicobacter pylori*-related idiopathic thrombocytopenia, *EMHJ* 2013.19 (7):676-681.

21- Vlachaki E., Agapidou A.,Klonizakis P. *Helicobacter pylori* and Immune Thrombocytopenic Purpura. *Immuno-Gastroenterology* 2012.1(2): 104-10

22- Veneri D, Krampera M, Franchini M. High prevalence of sustained remission of idiopathic thrombocytopenic purpura after Helicobacter pylorieradication: along-term follow-up study. *Platelets. 2005*.16 (2):117 119.

23- Asahi A., Nishimoto T., Okazaki Y, *et al*.*Helicobacter pylori* eradication shifts monocyte Fc gamma receptor balance toward inhibitory FcgammaRIIB in immune thrombocytopenic purpura patients. *J Clin Invest2008*. 118(8):2939-49.

Michel M, Cooper N, Jean C, Frissora C.and B ussel JB. Does *Helicobacter pylori* initiate or perpetuate immune thrombocytopenic purpura*? Blood*.2004 103(3):890-896.

24- Takashashi T. et al. Molecular mimicry by Helicobacter pylori CagA protein may be involved in the pathogenesis of *H. pylori*-associated chronic idiopathic thrombocytopenic purpura. Br J Haematol.2004 ;124(1):91-6

25- Franchini M., Plebani M., Montagnana M., Veneri D. and Lippi G. Pathogenesis, laboratory, and clinical characteristics of Helicobacter pylori-associated immune thrombocytopenic purpura.*Adv Clin Chem 2010*.52:131-44.

*** the national center of hematology**
**** almustansiriya medical college**