Usefulness of Helicobacter Pylori Eradication for Platelet Recovery in Egyptian Idiopathic Thrombocytopenic Purpura Patients

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Abstract: Background: Recent studies have shown a relationship between Helicobacter pylori (H. pylori) and idiopathic thrombocytopenic purpura(ITP). **Objectives:** To clarify the relation between H. pylori and ITP, determine its prevalence in this disease and to evaluate the effect of its eradication on platelet recovery. **Subjects and methods:** 65 adult patients with ITP (platelet count < 100 x 10^3/µl) were investigated for the presence of H. pylori infection and its eradication by H pylori stool antigen (HpSA) enzyme immunoassay method (EIA) . H. pylori positive patients received standard triple therapy for seven days to eradicate infection. Platelet counts were monitored every 2 weeks and assessed 6 months after the end of H. pylori eradication therapy. Uninfected patients underwent immunosuppressive therapy and their platelet counts were followed up for the same duration. **Results:** 45/ 65 ITP patients, were H. pylori positive. They were significantly older and showed longer disease duration than H. pylori negative patients .There was significant increase in platelet count in both group after treatment and this increase was significantly higher in H. pylori positive group than negative one. Out of the 45 infected patients who received treatment, H. pylori was successfully eradicated in 39 patients. In 21 (53.8%) of these patients , significant good platelet response was detected when compared with unsuccessfully treated and H. pylori negative patients. **Conclusion:** Eradication of H. pylori infection led to good platelet response in ITP patients. Therefore, search for this infection must be attempted in ITP patients at diagnosis which will allow a good non immunosuppressive option for some of them.

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1. Introduction:

Idiopathic thrombocytopenic purpura (ITP), also known as primary immune thrombocytopenic purpura is an acquired disease of both children and adults. It is defined as isolated thrombocytopenia with no clinically apparent associated conditions or causes of thrombocytopenia. So, its diagnosis relies on the exclusion[(1)]. The term idiopathic was coined because in the majority of cases the underlying cause was unknown. Recently, the list of etiologies has been steadily increasing, so, the term "idiopathic" is becoming obsolete, increasingly replaced by "immune" thrombocytopenic purpura[(2)]. Helicobacter pylori (H. pylori) is a gram-negative microaerophilic bacterium that colonizes the stomachs of over half the human population. It is the predominant agent of active chronic gastritis, gastric and duodenal ulcers. Also, it is a cofactor in the development of both adenocarcinoma and mucosal associated lymphoid tissue lymphoma[(3)]. Several studies have investigated the relationship between H. pylori and extra-gastroduodenal disorders. It is reported that it has been implicated in various autoimmune disorders[(4)]. H. pylori infection is driven by urease, flagella, and adhesions. Virulence factors such as CagA and VacA play roles in colonization and infection. Other virulence factors are H. pylori neutrophil-activating protein (HP-NAP) and cell-wall lipopolysaccharide (LPS)[(5-7)]. The role of H. pylori in the development of ITP is not yet known. Many hypotheses have been proposed to address the mechanisms by which H. pylori causes ITP. Platelet-associated immunoglobulin G, CagA, LPS etc., have all been reported to play a role in platelet apoptosis[(8,9)]. Since partial or even complete remission of thrombocytopenia has been recorded in some ITP patients after eradication of H. pylori it has been suggested that H. pylori may contribute in the pathogenesis of this disease[(10)]. Most studies of H. pylori and ITP are from Japan, Spain and Italy[(11)] To date the therapeutic option of H. pylori which is simpler and safer than immune-suppressives and splenectomy hasn't been carefully investigated in Egyptian ITP patients. This study was designed to clarify the relation between H. pylori and ITP, determine its prevalence in this disease and to evaluate the effect of its eradication on platelet recovery.

2. Study design:

Sixty five ITP patients (25 males and 40 females) were included and studied in Zagazig

University hospitals. ITP was diagnosed according to the American Society of Hematology (ASH) guidelines[1], based on thrombocytopenia (platelet count < 100 x 10^3/μl) with normal bone marrow or showing megakaryocytic hyperplasia. Secondary ITP caused by drugs, viral infection and collagen disease were excluded.

H. pylori infection was documented by detecting H. pylori antigens in stool specimens through Helicobacter pylori stool antigen (HpSA) enzyme immunoassay method (EIA)[12] and whenever possible, by histo-pathological examination using (Giemsa stain) for specimen obtained by an upper gastrointestinal endoscopy.

The stool sample from each patient was stored at 2-8°C for up to 24 hours or at -70°C if prolonged storage was required till the completion of a test batch. Thawing of the specimens was done by keeping them at room temperature for 1 hour. Premier Platinum HpSA plus kit (Meridian Diagnostic, Cincinati, Ohio. USA) was used for stool antigen detection as per manufacturer instructions. The test was performed in four steps:-

1)Specimen processing : A stool sample measuring 5-6 mm diameter was diluted in 200 μl of sample diluent and mixture was vortexed for 15 seconds. A total of 50 μl of the processed samples and equal volume of positive and negative controls were added to the appropriate micro-wells of the enzyme immune-assay (EIA) plate.
2)Sample-enzyme conjugation and incubation: A drop of enzyme conjugate was added to the wells and contents were firmly mixed for 30 seconds. The wells were sealed and incubated at 22-27°C for one hour. The contents of the wells were washed with buffer for five times.
3)Substrate incubation :Two drops of substrate were then added to each well and the plates were again incubated for 10 minutes at 22-27°C. A drop of stop solution was added to each well and mixed for 30 seconds.
4)The absorbance at 450 nm was immediately measured using a DaVinci (bioMérieux, France) microplate reader and were interpreted as positive if the optical density was more than 0.16 at wave length of 450 nm.

All infected patients gave a written consent ,immune-suppressives were stopped (if used by any) for one month and treated for 7 days with standard triple therapy (lansoprazole, 30 mg, clarithromycin 200 mg and amoxicillin 750 mg all twice daily)[13]. Eradication was confirmed by H. pylori stool antigen (HpSA) one month after completion of therapy. After completion of triple therapy infected patients were subdivided into successfully treated patients (eradicated infection) and unsuccessfully treated patients (uneradicated infection) based on repeated H. pylori detection tests.

Platelet counts were monitored every 2 weeks and assessed 6 months after the end of H. pylori eradication therapy. Uninfected patients underwent immunosuppressive therapy and their platelet counts were followed up for the same duration.

Rise of platelet count to normal value (150 – 450 x 10^3/μl) was considered as a complete response (CR), while increase of the count to less than 120 x 10^3/μl or 30 x 10^3/μl above the baseline count was considered partial response (PR)[14].

ITP patients whose platelet count didn't rise after H. pylori eradication or immunosuppressive therapy underwent splenectomy.

Statistical analysis:

Statistical analysis was done using the SPSS version 10.0. Data are represented as Mean±SD. Unpaired student t-test, fisher exact probability test and chi-square test, were used when appropriate .P<0.05 considered to be statistically significant in all tests.

3. Results:

Data were collected, summarized, analyzed and presented in the following tables:

Forty five (69.2%) patients were H. pylori positive (infected) (21 males and 24 females) with mean age 52 years (39-72 years), the remaining 20 patients (4 males and 16 females) were H. pylori negative (uninfected) with mean age 40.5 years (27-65 years), which is statistically significant different between the 2 groups (P<0.004). There was no statistically significant difference between both groups regarding the baseline platelet count (at the beginning of the study), mean values were 55.2 (11-92) x 10^3/μl and 56.7 (19-99) x 10^3/μl, respectively with P>0.05.

Disease duration was significantly shorter in H. pylori negative than H. pylori positive patients with mean values of 4.5 (4-10) and 8.2 (6-14) months, respectively. All previous data are shown in table (1).

Change in platelet count and comparison between its values for the H. pylori positive patients (before eradication therapy and after its completion) and for the H. pylori negative patients at the beginning of treatment and at the end of the same duration of treatment are represented by table (2) showing highly significant (P<0.0001) of both. Also, by the same table the difference between the mean values of platelets count for the two groups after completion of therapy is shown to be significant.

Infection in 39/45 (86.7%)of infected patients was successfully eradicated by completion of triple therapy, this subgroup is compared to those with

unsuccessfully eradicated infection [6/45 (13.3%)] and H.pylori negative patients regarding response of platelet count in table (3)which shows significant higher percentage (53.8%) of patients with good response among those with successfully eradicated infection than either those with unsuccessfully eradicated infection(0%) and H. pylori negative patients(35%) .

Table (1): Characteristics of H. pylori +ve and H.pylori –ve groups

Item \ Group	H. pylori +ve group (N:45)	H. pylori -ve group (N:20)	Test of significance	P
Age Mean±SD (Range)	52±16 (39-72)	40.5±10.2 (27-65)	t=2.95	0.004*
Sex Male/ Female	21/24	4/16	χ^2=3.11	0.078
Platelets count X10^3/ul Mean±SD (Range)	55.2±12 (11-92)	56.7±19 (19-99)	t=0.385	0.7
Disease duration	8.2±2.1 (6-14)	4.5±1.6 (4-10)	t=7.015	0.0001*

Table(2): Comparison between platelet count before and after therapy among studied groups.

Platelets count \ Group	H. pylori +ve group (N:45)	H. pylori -ve group (N:20)	t-test	P
Before treatment Mean±SD X10^3/ul (Range)	55.2±12 (11-92)	56.7±19 (19-99)	0.385	0.7
After treatment Mean ±SD X10^3/ul (Range)	230±20.2 (150-270)	186.7±10.7 (159-205)	9.014	0.0001*
t-test	49.9	26.7		
P	0.0001*	0.0001*		

Table(3): Response to therapy regarding infection eradication and platelet recovery among the studied groups.

Platelets count response \ H. pylori eradication response	H. pylori +ve group (N:45)		H. pylori -ve group (N:20)	P
	Successful eradication (N:39)	Unsuccessful eradication (N: 6)		
Good response	21/39 (53.8%)	0/6 (0%)	7/20 (35%)	0.02*
Partial response	4/39 (10.3%)	1/6 (16.7%)	3/20 (15%)	0.6
No response	14/39 (35.9%)	5/6 (83.3%)	10/20 (50%)	0.09

4. Discussion

Helicobacter Pylori has been considered for years as the only etiological agent of gastritis, peptic ulcer, gastric cancer and mucosa associated lymphoid tissue lymphomas[15]. Also, it has been found to be associated with a number of autoimmune disorders[16].

Globally, the prevalence of H. pylori infection in developing countries is markedly higher than that in developed countries[17,18].

Idiopathic thrombocytopenic purpura (ITP) is the most common autoimmune mediated hematological disorder. Its etiology, pathogenesis and molecular receptor targets remain unclear[11]. There is growing evidence of an association between H pylori eradication and platelets recovery in patients with ITP[19].

Aiming to participate in clarification of the relation between H. pylori and ITP, this study was carried out on 65 ITP patients among whom the

frequency of H. pylori infection was 69.2% (45/65) compared to 92% in Korea[3], 71% in Span[20], 62.5% in Japan[21], 62.7% in Italy[22], 56.3% in Australia[11], 29% in France[23] and down to lower rate (22%) in USA[4]. This variation in frequency of H pylori infection among ITP patients in these comparable studies may reflect the variation in ages of these studied groups of patients and give an impression about the potential regional variation of the prevalence of H pylori infection.

Recently, Semple and colleagues[24] demonstrated that in the presence of antiplatelet antibodies, the LPS of Gram negative bacteria can significantly enhance Fc-dependent platelet phagocytosis. 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[8].

Regarding age, in this study, H. pylori–infected patients were found to be significantly older (mean age:52 years) than H. pylori–uninfected (40.5 years) ones, which is consistent with that of similar studies[25,26]. This is not unexpected, as the prevalence of H. pylori infection in the general population increases with increasing age[27,28].

In respect to the mean duration of the thrombocytopenia, it was significantly longer in H. pylori-positive than that of H pylori negative patients (8.2 months versus 4.5 months) which was in agreement with that reported in different studies[29,30]. In contrast, as regard to other characteristics, such as sex and platelet count at the baseline all series that were reviewed failed to detect significant differences which is the case of this study.

In this study, data lend further support to a relationship between H. pylori infection and ITP as the platelet response of 64.1% (CR and PR) was noted after the eradication of H. pylori infection, whereas the corresponding rate was reported to be 50% by Emilia et al [31], 63.2% by Kohda et al [21] and 72.72% by Gasbarrini et al [32]. Moreover, 46.2% of the present study responders achieved CR versus 33.3% and100% of eradicated patients in reports by Emilia et al [31] and Gasbarrini et al [32] respectively. In contrast other studies refuted a significant association between of H. pylori infection and ITP[11] for example, a prospective study in the USA found that only 1/14 ITP who responded to H. pylori eradication had a rise in platelet count[4].

Variety in the rate of platelet response to bacterium eradication may be related either to the variability of host immune state (including HLA allele pattern , cytokines and chemokines produced in gastric mucosa in response to H. pylori infection) or to the bacterium's high genetic diversity, ie, to the existence of different H. pylori strains with possibly different pathogenic potential[33].

The response of the platelet count was also observed in one patient of six H. pylori– infected ITP patients who had unsuccessfully eradicated the infection in the present study. It has been advanced that the increased platelet count in patients who failed the H. pylori eradication or in those who received proton pump inhibitor monotherapy could have been mediated through a reduction in the quantity of H. pylori and/ or a bacteriostatic effect of the regimen[34].

Interestingly, the response of the platelet count was observed significantly increased after eradication therapy in H. pylori positive patients (mean 230)than H. pylori negative patients treated with immunosuppressives (mean 186.7) (P= 0.0001) . This finding could be explained in several ways, including an immune-modulatory effect of macrolides that is separated from the bacteriostatic effect[35].

In conclusion, considering the low costs, the noninvasiveness of diagnostic method, and much less toxicity and hazards of eradication therapy compared to standard ITP therapy (steroids or splenectomy), the assessment of H.pylori infection and use of its eradication therapy should be attempted in ITP.

Further studies are recommended on larger group of patients to fully ascertain the role of H pylori in ITP and for longer duration of follow up to assess the rate of relapse among the recovered cases and identify factors that may assist in selecting ITP patients who are more likely to respond to therapy.

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