Eur J Clin Microbiol Infect Dis (2016) 35:1171–1176
DOI 10.1007/s10096-016-2650-8

ORIGINAL ARTICLE

Impact of Helicobacter pylori eradication on refractory thrombocytopenia in patients with chronic HCV awaiting antiviral therapy

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Received: 28 December 2015 / Accepted: 14 April 2016 / Published online: 14 May 2016

Abstract The possibility of delaying treatment of HCV due to severe thrombocytopenia is challenging. This study aimed to detect the prevalence of active helicobacter infection as a claimed cause of thrombocytopenia in a cohort of Egyptian patients with chronic active HCV awaiting combined antiviral therapy. The study included 400 chronic HCV patients with thrombocytopenia. Laboratory investigations included liver function tests, real time quantitative PCR, reticulocytic count, ESR, ANA, bone marrow aspiration, measurement of anti-helicobacter antibodies, and helicobacter stool antigen. Positive cases for active *H. pylori* were given the standard triple therapy for 2 weeks. Helicobacter stool antigen was detected 4 weeks after termination of therapy and the change in platelet count was detected 1 month after eradication. A total of 248 out of 281 seropositive patients for *H. pylori* (88.3 %) showed positive stool antigen ($p=0.01$). Eradication was achieved in 169 (68.1 %) patients with platelet mean count $114.9 \pm 18.8 \times 10^3/\mu l$ with highly significant statistical difference from pretreatment value ($49.7 \pm 9.2 \times 10^3/\mu l$, $p=0.000$). Seventy-nine patients were resistant to conventional triple therapy and given a 7-day course of moxifloxacin-based therapy; 61 patients responded (77.1 %) with mean platelet improvement from $76.4 \pm 17.4 \times 10^3/\mu l$ to $104.2 \pm 15.2 \times 10^3/\mu l$ ($p=0.000$). The non-responders showed no improvement in their platelet count (74.6 ± 20.5 vs. $73.6 \pm 15.3 \times 10^3/ul$, $P=0.5$). Eradication of active *H. pylori* in HCV augments platelet count and enhances the early start of antiviral therapy.

Abbreviations

AVACT	Anti viral combination therapy
DAA	Direct acting antivirals
HCC	Hepatocellular carcinoma
ITP	Idiopathic thrombocytopenic purpura
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
SVR	Sustained virological response

Introduction

H. pylori is a Gram-negative microaerophilic bacterial species that colonizes the human stomach, it exists in about 50 % of the global population and is implicated in the development of gastritis, mainly the atrophic type with the possibility of metaplasia, so it is considered a risk factor for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma [1]. The lipopolysaccharide of *H. pylori* may induce autoantibodies that aggravate the atrophy of gastric mucosa [2, 3].

The link between *H. pylori* infection and chronic idiopathic thrombocytopenic purpura (ITP) was first described by an Italian group in 1988 who reported a significant increase in the platelet count after successful eradication of *H. pylori* with a persistent increase in platelet counts in more than 50 % of the patients [4, 5].

H. pylori may contribute to the pathogenesis of ITP due to the molecular mimicry between the highly antigenic Cag A protein and platelet surface antigens glycoproteins (GP) GPIIb/IIIa or GP Ib with the production of anti-platelet autoantibodies [6, 7].

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The prevalence of thrombocytopenia in chronic liver disease ranges from 15 to 70 % according to the severity of fibrosis and portal hypertension [8]. Mechanisms of thrombocytopenia in patients with chronic HCV include advanced hepatic fibrosis [9], hypersplenism with redistribution of blood to the spleen [10], bone marrow suppression caused by HCV infection [11], immune dysfunction caused by binding of HCV to platelets which induces the development of hapten-like neoantigens on the platelet surface [12] and decreased thrombopoietin levels or activity [13].

HCV-related thrombocytopenia associated with the risk of major bleeding is defined as a platelet count of 20,000 to < 70,000/ul [14]. With previous regimens which included pegylated interferon, treatment was contraindicated if platelet counts were below 75,000–100, 000 cells/μl [15]. In the era of direct-acting antivirals (DAA), the lower accepted limit of platelet count is 50, 000 cells/μl according to Egyptian national committee guidelines for control of viral hepatitis.

Thrombocytopenia in HCV-related advanced liver disease has a deep impact on its management due to the possibility of delaying treatment till improvement and this may reduce the chance of SVR. Thus, the aims of the present work were to investigate the prevalence of active *helicobacter pylori* in a cohort of Egyptian patients with HCV-related advanced liver disease who were awaiting combined anti-viral therapy but postponed due to severe thrombocytopenia, and to evaluate if its eradication will improve platelet counts and hasten the start of therapy.

Methods

Patient selection

The study included 400 patients with advanced liver disease due to chronic active HCV who were planned to receive antiviral combination therapy (AVCT) but they were postponed due to severe thrombocytopenia. They were evaluated in the gastroenterology unit of the Internal Medicine Department at the Zagazig University Hospital in Egypt, which is a tertiary referral center, in the period extending from October 2013 to February 2015.

The diagnosis of chronic active HCV was based on positivity of HCV antibodies, real-time PCR, and elevated liver enzymes. Thrombocytopenia is defined as a platelet count less than 150,000 /μl, and in our study a count less than 50,000 /μl that postponed the start of treatment was included.

Patients were excluded if they had HCC, HBV and features of severe liver dysfunction such as ascites or hepatic encephalopathy, drug-induced thrombocytopenia, ITP whether primary or secondary and thrombocytopenia due to hematological malignancy.

A control group included 200 patients with chronic HCV, and thrombocytopenia without concomitant *H. pylori* infection was compared to the study patients.

Thorough medical history and clinical examination

Signs of liver cirrhosis and portal hypertension, liver cell failure and generalized lymphadenopathy were reported. The Child-Pugh score was calculated for each patient.

Laboratory evaluation

Laboratory evaluation included routine liver and kidney function tests, PT, PTT, INR, HCVAB, HBsAg, real-time quantitative PCR for HCV (COBAS Ampliprep/Taqman HCV monitor, with the detection limit 15 IU/ml; Roche Diagnostic Systems), reticulocyte count, ESR, antinuclear antibodies (ANA) and bone marrow aspiration.

Detection of helicobacter infection

Measurement of anti-helicobacter antibodies (AB)

Serum samples were tested for the presence of IgG antibodies against *H. pylori* using Accu-Tell® Rapid H. pylori test (AccuBioTech Co., Ltd, USA). A positive result does not distinguish between active infection and colonization by *H. pylori*.

Helicobacter stool antigen

One-step lateral flow immunoassay for qualitative detection of helicobacter stool antigen (DRG International Inc., USA).

H. pylori eradication protocol

Cases with active *H. pylori* infection were given the standard triple therapy for 2 weeks including lansoprazole 30 mg twice daily, amoxicillin 1 g twice daily, and clarithromycin 500 twice daily together with folic acid 1 mg daily and vitamin B complex injection and compared to the control group who received only folic acid and vitamin B complex. Helicobacter stool antigen was detected 4 weeks after termination of therapy and then the patients were followed for 1 month following eradication to detect the change in platelet count.

Statistical analysis

Data were statistically analyzed using SPSS version 21. Results were expressed as mean ± SD. Categorical

Table 1 Baseline demographic and laboratory characteristics of the study and control patients

Variable	Study patients ($N=400$)	Control group ($N=200$)	P-value
Age (years)	50.6 ± 8.4	49.4 ± 7.5	0.1
Male/Female	247/ 153	124/76	0.7
HB (gm/l)	9.2 ± 1.1	10.5 ± 0.8	0.001
Platelets (/μl)	65.6 ± 22.7	82.6 ± 18.6	0.002
INR	1.8 ± 0.8	1.6 ± 0.6	0.002
Albumin (g/dl)	3.3 ± 0.3	3.2 ± 0.5	0.01
T. bilirubin (mg/dl)	1.7 ± 0.1	1.6 ± 0.2	0.01
AST (IU/L)	74 ± 20	54.4 ± 15.3	0.001
ALT (IU/L)	66 ± 15	52 ± 15	0.001
AFP (ug/l)	11.6 ± 0.6	8.9 ± 3.2	0.001
Creatinine (mg/dl)	1.4 ± 0.2	1.1 ± 0.3	0.001
RNA (KIU/L)	578 ± 14.3	354 ± 18.5	0.001

INR international normalized ratio, *AFP* alpha fetoprotein, *KIU* kilo international unit

variables were analyzed using the $\chi 2$ test and continuous variables were analyzed using the Student's *t*-test. Associations determined by correlation analysis were expressed as a Pearson's correlation coefficient (r). Multivariable linear regression analysis was used to detect independent variables. Comparing platelet count before and after triple therapy was done by paired *T* test. $P<0.05$ was considered to be statistically significant.

Results

Baseline laboratory data of the study patients are shown in Table 1; there was male predominance and the mean platelet count was 65.6 ± 22.7× 103/μl.

Prevalence of active helicobacter infection in the study patients

A total of 119 patients (29.8 %) were negative for anti-helicobacter antibodies; however, 281 patients (70.2 %) were seropositive and helicobacter stool antigen was positive in 248 of them (88.3 %), which was statistically significant ($\chi 2=6.077$, $P=0.01$).

The patients were classified into two groups: HCV with active *H. Pylori* which included 248 patients and HCV without active *H. Pylori* which included 152 patients. The latter group included 119 patients who were *H. Pylori* seronegative and 33 patients who were seropositive with absent *H. Pylori* stool antigen as shown in Table 2.

There was a highly significant statistical difference between both groups as regards hemoglobin level, which was

Table 2 Demographic and laboratory characteristics of the study patients according to active *H. pylori*

Characteristic	HCV with active *H. pylori* ($n=248$)	HCV without active *H. pylori* ($n=152$)	P-value
Age	51.1 ± 8.1	50 ± 8.7	0.8
M/F	154/ 94	93/ 59	0.8
HB gm/l	8.6 ± 0.7	9.5 ± 1.2	0.001
Platelets /μl	58.5 ± 18.9	78 ± 26	0.001
INR	1.75 ± 0.7	1.8 ± 0.73	0.4
Albumin g/dl	3.4 ± 0.4	3.5 ± 0.4	0.05
T.bilirubin mg/dl	1.7 ± 0.1	1.6 ± 0.1	0.5
AST IU/L	69 ± 18	72 ± 20	0.9
ALT IU/L	62 ± 11	66 ± 15	0.7
AFP ug/l	13.7 ± 5.7	8.1 ± 3	0.000
Creatinine mg/dl	1.5 ± 0.2	1.3 ± 0.1	0.001
RNA KIU/L	835 ± 14	577 ± 24	0.000

H. pylori infection

Table 3 Effect of eradication of *H. pylori* on platelet count in patients with HCV

Triple therapy	Response	No response	*P*-value
1- Initial ($n=248$)			
- Number (%)	169 (68.1 %)	79 (31.9 %)	**0.000**
- Pretreatment platelets	49.7 ± 9.2	77 ± 21	0.89
- Post-treatment platelets	114.9 ± 18.8	76 ± 18	**0.0001**
- Paired *T* test	**0.000**	**0.4**	**0.0001**
2- Retreatment ($n=79$)			
- No (%)	61 (77.1 %)	18 (22.9 %)	**0.000**
- Pretreatment platelets	76.4 ± 17.4	74.6 ± 20.5	0.84
- Post-treatment platelets	104.2 ± 15.2	73.6 ± 15.3	**0.0001**
- Paired *T* test	**0.000**	**0.5**	

Values in italics indicate statistical significance

lower, and also serum creatinine, AFP, and HCV RNA levels, which were higher in the group with positive *H. pylori* infection ($p<0.05$).

The patients who had active helicobacter infection were given the standard triple therapy according to the planned protocol. A total of 169 (68.1 %) patients showed eradication of helicobacter denoted by absent *H. Pylori* fecal antigen 1 month after termination of therapy. One month later their platelet count mean value became $114.9 \pm 18.8 \times 10^3$ /μL with a highly significant statistical difference from pretreatment value ($49.7 \pm 9.2 \times 10^3$/μL, $p=0.000$). The non-responders showed trivial or no improvement in their platelet count ($76 \pm 18 \times 10^3$/μL vs. $77.1 \pm 21 \times 10^3$ /μL, $p=0.4$).

Seventy-nine patients were resistant to conventional triple therapy, and they were given a 7-day course of 400 mg moxifloxacin once daily, 1000 mg amoxicillin twice, and 20 mg esomeprazole twice [16]. Sixty-one patients were responders (77.1 %) with improvement of their mean platelet count from ($76.4 \pm 17.4 \times 10^3$/μL to $104.2 \pm 15.2 \times 10^3$ /μl, $p=0.000$). The non-responders showed no improvement in their platelet count (74.6 ± 20.5 vs. $73.6 \pm 15.3 \times 10^3$, $P=0.5$) as shown in Table 3.

One hundred twenty-six out of 248 patients (51 %) who presented with HCV and active *H. pylori s*howed severe thrombocytopenia (≤50.000/ul) with subsequent delay in initiation of antiviral therapy; 110 of them were responders to triple therapy (87.3 %) with improvement of platelet count from 45 ± 4.3 to $115.2 \pm 20 \times 10^3$/ul.

Fifty-five out of 85 patients (34.3 %) who showed moderate thrombocytopenia (51–80.000 /ul) were responders to triple therapy (64.7 %) with an improvement of their platelet count from 56.6 ± 3.1 to $113.5 \pm 17.6 \times 10^3$/ul.

Four out of 37 (10.8 %) patients who showed mild thrombocytopenia (81–150.000 /ul) were responders to treatment with an improvement of platelet count from 90 ± 2.3 to $124.5 \pm 17 \times 10^3$ /ul as shown in Table 4, Fig 1.

Laboratory data from the control group ($n=200$) (119 patients from the study patients and 81 patients recruited from the gastroenterology outpatient clinic) are shown in Table 1.

Adding conventional therapy as folic acid and vitamin B complex showed no significant improvement in platelet count (82.6 ± 18.6 vs $87.4 \pm 11.3 \times 10^3$ /ul, $p=0.53$).

Platelet count was correlated with age ($r=-0.395$, $p=0.000$), sex ($r=-0.316$, $p=0.000$), INR ($r=-0.178$, $p=0.000$), albumin ($r=0.103$, $p=0.039$), and creatinine ($r=-0.364$, $p=0.000$).

Helicobacter stool antigen was correlated with hemoglobin level ($r=0.109$, $p=0.029$), AFP ($r=0.471$, $p=0.000$), and anti helicobacter antibodies ($r=0.831$, $p=0.000$).

The positivity of helicobacter stool antigen in chronic HCV patients complicated with thrombocytopenia was independently associated with hemoglobin level ($\beta=0.060$, $P=0.03$), AFP ($\beta=0.094$, $P=0.003$) and anti-helicobacter antibodies ($\beta=0.785$, $P=0.000$).

Discussion

Patients with HCV-related liver cirrhosis are frequently exposed to gastric and duodenal mucosal disorders; however, little information is available on the relationship of these lesions with *H. pylori* infection [17].

Platelet count less than 75,000 reflects the presence of portal hypertension and may postpone the initiation of anti viral therapy. The study showed that the rate of *H. pylori* seropositivity in patients with chronic HCV was 70.3 %; however, the rate of positive *H. pylori* stool antigen among the seropositive patients was 88.2 %.

The present study aimed to detect the prevalence and the possible role of *H. Pylori* in the development of thrombocytopenia. All the patients with proven active

Table 4 The response rate after triple therapy among patients with different grades of thrombocytopenia

Thrombocytopenia with *H. pylori* + ve ($n=248$)	Response	No response	*P*-value
Severe (≤50.000/ul) ($n=126$)	110 (87.3 %)	16 (12.7 %)	$\chi2=43.3$, $p=0.000$
Moderate (51–80.000/ul) ($n=85$)	55 (64.7 %)	30 (35.3 %)	$\chi2=0.71$, $p=0.4$
Mild (81–150.000/ul) ($n=37$)	4 (10.8 %)	33 (89.2 %)	$\chi2=65.9$, $p=0.000$

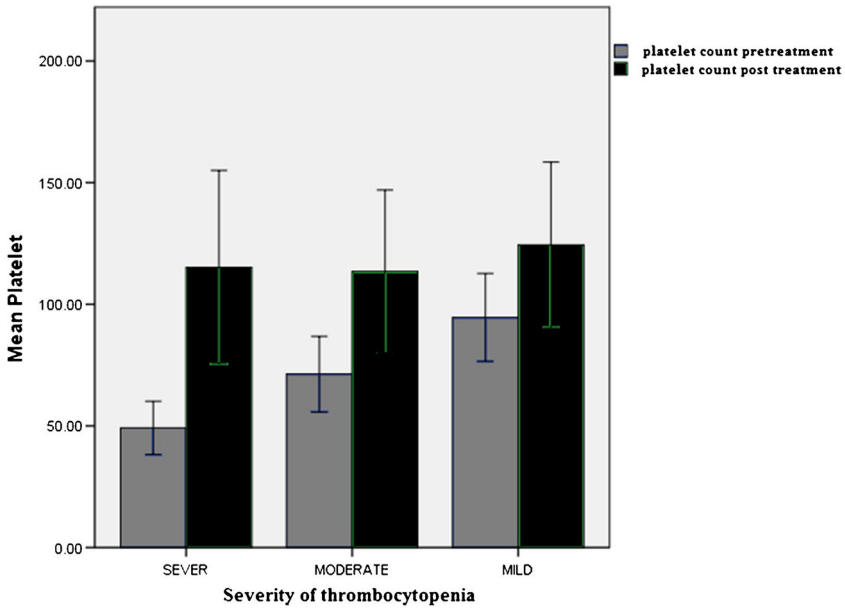

Fig. 1 Effect of treatment of *H. pylori* on platelet count according to severity of thrombocytopenia

infection were given the standard therapy and compared to the control group who were given only supportive therapy. A highly significant rise of platelet count was noticed in the former group after eradication of *H. pylori* ($n = 169$, 68.1 %); the non-responders ($n = 79$) were given another trial of moxifloxacin based therapy with eradication in 61 patients (77.1 %), and highly significant rise of platelet count was seen in the responders ($p = 0.000$).

One report showed that association of *H. pylori* infection with HCV did not influence the clinical course of disease [18]. Another report showed *that H. pylori* infection may reflect chronic liver damage by HCV infection [19].

The high prevalence of *H. pylori* in patients with chronic HCV could be explained by associated T cell dysfunction. It was proposed that *H. pylori* is implicated in the pathogenesis and progression of cirrhosis particularly in HCV and the involvement in hepatocellular carcinoma seems highly possible [20].

Recognizing and treating *H. pylori* in patients with HCV associated thrombocytopenia avoids the use of pharmacological agents with many side effects such as with steroids and danazol [21], precludes expensive treatments such as thrombopoietin agonists [22, 23], and avoids invasive maneuvers such as splenectomy and partial splenic embolization, which are risky in patients with advanced liver disease.

In conclusion, eradication of *H. pylori* when associated with HCV augments platelet counts, enhances the early start of antiviral combination therapy and avoids the use of highly expensive agents for thrombocytopenia.

Compliance with ethical standards

Funding The research is self-funded.

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

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional research committee and with the Helsinki Declaration and its later amendments.

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