Original Article

Association of Helicobacter Pylori infection with Idiopathic Thrombocytopenic Purpura

Kashif Hafeez Shaikh, Suhaib Ahmed, Muhammad Ayyub, Jaleel Anwar
Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan.

Abstract

Objective: To determine the association of Helicobacter pylori infection in patients presenting with idiopathic thrombocytopenic purpura (ITP).

Methods: From March 2007 to March 2008, thirty adult patients with ITP and 30 age and sex matched healthy controls were investigated for the presence of H. pylori infection by Helicobacter pylori stool antigen (HpSA) an enzyme immunoassay (EIA) based method. The criteria for presence of H. pylori infection was a positive stool antigen test.

Results: H. pylori infection was found in 19 out of 30 patients with ITP (63.3%) which is well above the frequency of 13 out of 30 (43.3%) in controls. Calculated odds ratio was 2.25 which shows significant association of H. pylori infection with ITP.

Conclusion: The study confirms the existence of an association between H. pylori infection and ITP. Therefore the screening for H. pylori infection and an attempt to eradicate bacterium in positive cases seems appropriate in patients with ITP at diagnosis (JPMA 59:660; 2009).

Introduction

Idiopathic thrombocytopenic purpura (ITP) is a haematological disorder characterized by sensitization of platelets by autoantibodies leading to platelet destruction.[1] Although its cause remains unclear, ITP is associated with several diseases, including infections.[2-4] Helicobacter pylori (H. pylori) infection is the world's most common chronic infection in humans and is the cause of most gastritis cases.[5,6] Recently, H. pylori has been found to be associated with ITP and its eradication has shown improvement in platelet count.[7-10] Many interpretations for its pathogenetic mechanism have been suggested but the phenomenon is still unclear.[11] However, there are few observations which have been documented in this regard. One study, indicated that platelets are capable of interaction with leucocytes in initiating and maintaining an inflammatory process leading to formation of Platelet-granulocyte or platelet-only aggregates, under the surface epithelium in gastric mucosa biopsies of these patients. A decrease in a number of peripheral blood platelets can be caused by the effect of platelet and platelet-granulocyte aggregations in response to H. pylori infection.[12] Another relevant observation is that H. pylori strains are highly diverse antigenically and strain diversity is associated with variability in host immune response with some patients having thrombocytopenia while others are spared.[13] Recent studies suggest a cross reaction between antibodies against H. pylori Cag A (cytotoxin associated gene A) protein and platelet antigens as the pathogenetic mechanism.[14] The recognition of H. pylori infection as a cause of ITP is important because steroids, which are the main stay of treatment for ITP, can aggravate H. pylori infection and associated symptoms.[15] We have determined the frequency of H. pylori infection in patients with ITP to ascertain significant association with ITP.

Patients and Methods

The study included 30 adult patients with ITP identified by Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi between March 2007 and March 2008. Control group of 30 healthy individuals was age and sex matched.

ITP was defined according to guidelines of the American Society of Haematology[16] as idiopathic thrombocytopenia (platelets < 100 x 10^9/l) persistent for more than 6 months, with normal or increased megakaryocytes in the bone marrow, and when other causes such as Hepatitis C virus and HIV infections, drugs, lymphoproliferative disorders, autoimmune disorders, and pseudothrombocytopenia had been excluded.

H. pylori infection was documented by detecting H. pylori antigens in stool specimens through Helicobacter pylori stool antigen (HpSA) enzyme immunoassay method (EIA).[17]

The stool sample from each patient was stored at 2-8°C for upto 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 kit (Meridian Diagnostic, Cincinati, Ohio) was used for stool antigen detection as per manufacturer instructions. The test was performed in following 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 immuno 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 results were analyzed spectrophotometrically by SunRise EIA Reader (Tecan, Sweden) and were interpreted as positive if the optical density was more than 0.16 at wave length of 450 nm.

Results

A total of 30 adult patients with ITP were evaluated in the study along with equal number of age and sex matched healthy controls. Among, 30 patients with ITP there were 17 females and 13 males, with a female to male ratio of 1.3:1. The frequency of H. pylori infection was found to be higher among females 12 (40.0 %) (95% CI = 22.5 - 57.5%) as compared to males 7(23.4 %) (95% CI = 8.2 - 38.5%). The mean age of H. pylori infected ITP patients was higher (34 years ± 17 years) than those without infection (26 years ± 8 years).The mean platelet count in patients with ITP was 36 x 10^9/l (± 31 x 109/l) while in healthy controls it was 255 x10^9/l (± 79 x 109/l). Among the patients with ITP it was similar between the H. pylori infected (35 x 10^9/l [± 30 x 10^9/l]) and non infected patients (38 x 10^9/l [± 35 x 10^9/l]). H. pylori infection was found in 19 out of 30 patients with ITP (63.6%) by HpSA stool antigen test (95% confidence interval [CI] = 33.8 - 80.5%). Thirteen out of 30 controls (43.6%) were also positive for H. pylori infection (95% CI = 25.6 - 61.0%) (Table).The calculated odds ratio was 2.25. It is more than the recommended cut off value of less than 1.0 which establishes a highly significant association of H. pylori infection with ITP.

Table: Frequency of Helicobacter pylori infection in patients with and without idiopathic thrombocytopenic purpura.

Risk	Patients with ITP (Cases) n = 30		Patients without ITP (Controls) n = 30	
	Frequency n (%)	95% Confidence interval (%)	Frequency n (%)	95% Confidence interval (%)
H. pylori Positive	19 (63.3)	33.8 - 80.5	13 (43.3)	25.6 - 61.0
H. pylori Negative	11(36.7)	19.5 - 53.9	17 (56.7)	38.9 - 74.4

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.[5] More recently it has been found to be associated with a number of autoimmune disorders including ITP.[18] The studies reported in literature have found a high frequency of H. pylori infection in patients with ITP and in most of them an increase in platelet count was observed after H. pylori eradication.[7-10]

This study included 30 patients with ITP along with equal number of age and sex matched controls. A high frequency of H. pylori infection in patients with ITP 19/30 (63.3%) was found which was well above the frequency in control individuals 13/30 (43.3%). The calculated odds ratio (2.25) established significant association between H. pylori and ITP. The low frequency of H. pylori infection in control group was seen, when compared to literature for developing countries,[19] this can be explained by higher socioeconomic status of the control individuals. This is in accordance with the impact that socioeconomic status has on the prevalence of H. pylori infection.[20] When assessed by gender, women (40.0%) showed a higher rate of infection than men (23.4%) which may be due to higher prevalence of ITP observed in women.[21] When assessed by age, mean age is higher for the infected patients (34 years) than noninfected (26 years) ones, which can be explained by the fact reported in the literature that prevalence of H. pylori infection increases with increasing age.[22]

The studies reported in literature have found a high frequency of H. pylori infection in patients with ITP and in most of them an increase in platelet count was observed after H. pylori eradication.[7-10] Worldwide, this association has been found in other (27) studies also.[7-10,23,24] The consolidated analysis of 27 reported studies worldwide shows that from a total of 1740 patients with ITP, 1144 (65.7%) were infected with H. pylori. This shows that the frequency of H. pylori infection in our study 19/30 (63.3%) is similar to the studies reported worldwide. In contrast, studies conducted in France[23] and USA,[24] have reported a low frequency of H. pylori infection. This could be explained by the low prevalence of H. pylori in local population.

The present study is the first of its kind in Pakistan that finds an association between ITP and H. pylori infection. The results of this study suggest a strong association between H. pylori infection and ITP. Although, data must be interpreted with caution because of the variability in rate of H. pylori infection in different regions and generations.[19] Future studies, should take into consideration other possible factors that could influence the interaction between host (genetic factors, age) and infectious agent (prevalence, bacterial strains), particularly since the prevalence of H. pylori varies greatly from country to country.[19]

The discovery of H. pylori decades ago resulted in a dramatic paradigm shift that has led the scientific and medical community to revise the management of dyspepsia and gastric cancers.[5] Now accumulating evidence of an association between H. pylori infection and ITP is opening new doors for a group of patients with a chronic disease that is difficult to manage. Therefore, with increasing evidence, ITP can be considered as an infectious disease that can be reverted once the bacteria has been eradicated. In fact the guidelines on the management of H. pylori updated at the European Helicobacter study group third Maastricht consensus conference in March 2005 has recommended eradication of H. pylori in patients with ITP.[25] Future studies, should now concentrate on establishing a concrete role of H. pylori infection in ITP by eradication of H. pylori infection in patients with ITP and monitoring platelet recovery with long term follow up.

Conclusion

The study confirms the existence of an association between H. pylori infection and idiopathic thrombocytopenic purpura (OR = 2.25). Further investigations on a larger number of patients might allow a better definition of the true prevalence of H. pylori infection in patients with ITP. These positive results also justify placebo controlled trials for the assessment of effect of eradication of H. pylori in patients with ITP. Until the results of such trials are available, the screening for H. pylori infection and an attempt to eradicate bacterium in positive cases seems appropriate in patients with ITP at diagnosis.

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