ORIGINAL ARTICLE

Vitamin B_{12}, Serum Ferritin and Iron status in patients with and without *H. Pylori* infection

SAQIB SOHAIL[1], SHMYLA HAMID[2], IJAZ ANWAR[3], *SAIM KHAWAR[4], HAMID JAVED QURESHI[5]*

ABSTRACT

Background: *Helicobacter pylori* are coccoid or spiral shaped organisms 3 µm in length and 0.5 µm in diameter. Human stomachis the principal reservoir for*Helicobacter pylori*.It produces gastric and extragastric complications by producing Vac A and Cag A toxins.
Aim: To investigate the correlation of extragastric complications like reduction in vitamin B_{12}, iron and ferritin to that of *Helicobacter pylori infection*.
Methods: A total number of 90 subjects were divided into three groups A, B and C. Serum ferritin and vitamin B_{12} was estimated by chemilumiscence technique. Serum iron was assessed by calorimetric method.
Results: Serum vitamin B12 values (288.13±160.09, 262.53±130.79, and 261.83±135.35pmol/l) in groups A, B, and C, respectively did not show any significant difference (p >0.05). Also no difference was noted in serum iron concentration between groups A (102.04±87.81µg/dl), group B (104.91±34.93 µg/dl), and group C (108.93±2.13µg/dl). The serum ferritin values in group A (65.13±48.03 ng/ml), group B (60.55±42.07ng/ml), and group C (68.96±18.44ng/ml) were also statistically non-significant.
Conclusion: The results of our study reveal that *H. pylori* infection is not the cause of vitamin B_{12}, serum ferritin and iron deficiency.
Keywords: Serum ferritin, H. pylori infection, Vitamin B_{12}

INTRODUCTION

Helicobacter pylori are a spiral shaped microaerophilic gram negative bacteria. It is about 3 µm in length and 0.5 µm in diameter[1]. Reservoir of *Helicobacter pylori is* human stomach[2]. It survives by neutralizing gastric acidity and produces mucosal damage by producing Vac A and Cag A toxins[3,4].

H. pylori produce duodenal ulceration by producing more G cells in stomach antrum which secretes gastrin[5]. The resultant increased release of gastrin from G cells in stomach antrum produces two effects, first it increases parietal cell mass and secondly, increases acid production from parietal cells in the stomach to increase the amount of acid in the stomach. *H. pylori* also causes decreased inhibition of hydrochloric acid production by destroying D cells from the stomach antrum because D cells secrete somatostatin that causes inhibition of the gastrin. In turn, gastrin further increases acid secretion from the parietal cells[6]. *Helicobacter pylori* produces gastritis and gastric ulcers by producing inflammatory changes that damage the defensive mucosal barrier[7,8]. *Helicobacter pylori* associated gastritis is reported to result in many extra gastric complications like iron deficiency and iron deficiency anemia, vitamin B_{12} deficiency and megaloblastic anemia[9]. This study is planned to evaluate derangement in the serum B12, ferritin and iron in patients with H. pylori infection.

[1]Associate Professor Physiology, PGMI/Ameerud Din Medical College, Lahore
[2]Asstt Prof Physiology, Sh. Zayed Medical College, R Y Khan
[3]Asstt. Prof. Physiology, Nawaz Sharif Medical College, Gujrat
[4]Lahore Medical & Dental College, Lahore
[5]Principal &Prof. of Physiology, Akhtar Saeed Medical College, Lahore
Correspondence to Dr. Saqib Sohail Email: saqibsohail51@yahoo.com, Cell: 0300-4357314

MATERIAL AND METHODS

It was a cross sectional analytical study conducted at the University of Health Sciences, Lahore. Subjects having *Helicobacter pylori* infection with gastric symptoms and subjects having gastric symptoms without this infection were selected from the Services Hospital, Lahore.

A total number of ninety subjects including both male and female were selected by random non purposive sampling for the study. The subjects were between 15-60 years of age. They were divided into three groups. Each group comprised of thirty subjects. In Group 1, subjects having history of gastric symptoms and positive for *Helicobacter pylori* infection after determining serum *Helicobacter pylori* antibodies quantitatively and by carrying out biopsy based rapid urease test and histopathological examination were included in this group. Group 2 was composed of subjects with history of gastric symptoms without *Helicobacter pylori* infection while healthy subjects without gastric symptoms and

Helicobacter pylori infection were included in the group 3.

Subjects were excluded if they were found to be positive for intrinsic factor antibodies and had history of gastric surgery, blood transfusion, iron or vitamin B_{12} supplement intake, pregnancy, bleeding ulcer, menorrhagia and fever.

METHODS

An informed consent was taken from all the subjects after explaining the study purpose and procedure. A detailed history was taken from each subject and clinical examination was also carried out. Initial screening of subjects was carried out by performing quantitative enzyme immunoassay for *Helicobacter pylori* IgG. Gastric biopsy samples were collected from the subjects having symptoms of gastric disease through gastric endoscopy for the confirmation of *Helicobacter pylori* infection on the basis of rapid urease test and histopathological examination. Serum ferritin was estimated by chemilumiscence technique. Serum ferritin kit by Ortho-Clinical Diagnostics (Lot no. 1070) was used. Serum iron was measured by endpoint colorimetric method. The kit used for the measurement of iron was manufactured by Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom (Cat No. S1257). Serum vitamin B_{12} was estimated by chemilumiscence technique. Serum vitamin B_{12} kit by Ortho-Clinical Diagnostics was used (Lot no. 1330). Aeskulisa Intrinsic Factor EIA kit (Lot No. 08130) by Aesku Diagnostics, Germany was used for the quantitative and qualitative detection of IgG antibodies against intrinsic factor.

Data was analyzed by using standard SPSS version 16. Arithmetic mean and standard deviation of all the quantitative variables like iron, ferritin and vitamin B_{12} was determined. One way ANOVA was applied to determine the difference between groups and association between qualitative variables. The p value of less than 0.05 was considered statistically significant.

RESULTS

Serum vitamin B12 values (288.13±160.09, 262.53±130.79, and 261.83±135.35pmol/l) in groups A, B, and C, respectively did not show any significant difference (p >0.05). There was no significant difference in serum iron concentration between groups A (102.04±87.81µg/dl), group B (104.91±34.93µg/dl), and group C (108.93±52.13µg/dl). The serum ferritin values in group A (65.13±48.03ng/ml), group B (60.55±42.07ng/ml), and group C (68.96±18.44ng/ml) were significantly not different.

Fig. 1: Serum Vitamin B_{12} in groups A, B and C

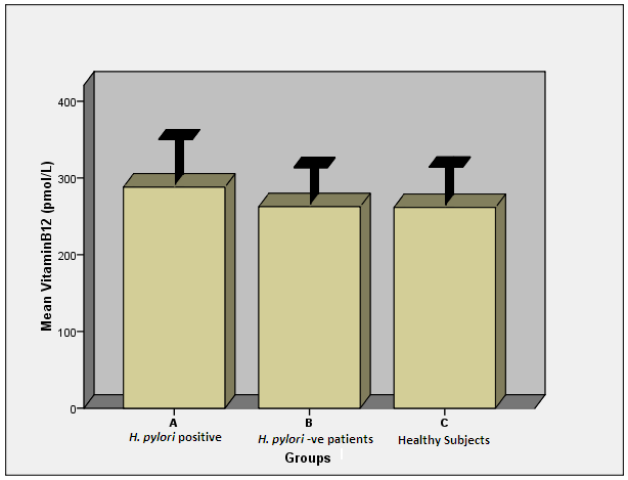


Fig. 2: Serum iron in groups A, B and C

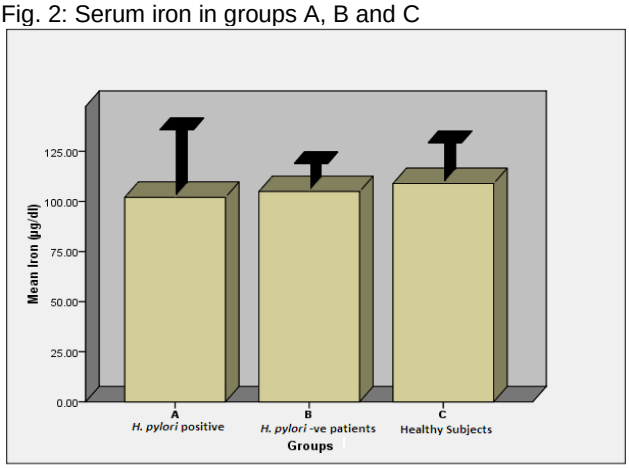
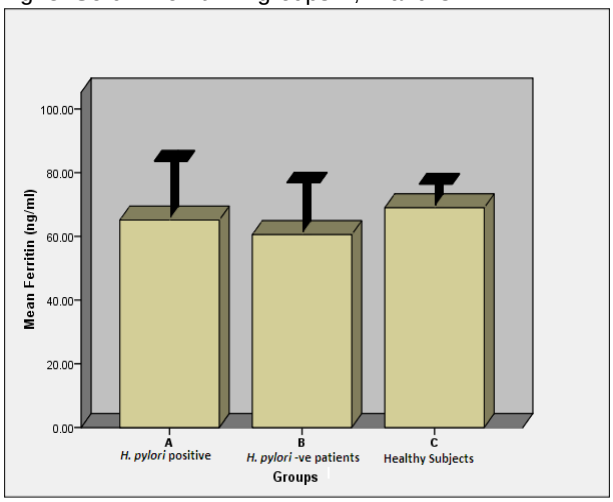


Fig. 3: Serum Ferritin in groups A, B and C



DISCUSSION

It is proposed that *H. pylori* causes vitamin B12 deficiency by inducing hypochlorhydria or achlorhydria by neutralizing the gastric acidity and reducing pepsin secretion but still the exact mechanism is not clear[10,11,12,13,14,].The exact mechanism of iron deficiency and anemia due to iron deficiency is not clear. The proposed mechanisms are that *H. pylori* first neutralize gastric acidity by producing ammonia, thus decreasing the gastric power to dissolve the dietary content of iron[12,15]. Secondly, *H. pylori* decreases the vitamin C content of the gastric juice which is required for the formation of soluble complexes with iron, and also required as a cofactor for the reduction of ferric to ferrous form [16]. Third, it utilizes dietary iron for its own use[11].

The results of our study did not show any significant variation between healthy subjects and patients. Literature review shows controversial picture about the association of *Helicobacter pylori* infection and iron deficiency anemia. No anemia or iron deficiency was revealed in *Helicobacter pylori* infected patients[17]. In the same way, a large sample survey conducted in Denmark revealed only iron deficiency but no effect on the hemoglobin; mean corpuscular volume that could indicate iron deficiency anemia[18].All the parameters of iron deficiency anemia like hemoglobin and mean corpuscular volume showed improvement after eradication therapy for *Helicobacter pylori* infection except the serum ferritin levels[19]. Asymptomatic *Helicobacter pylori* infection was not found to be associated with anemia or iron deficiency[20]. No significant difference was observed in hemodialysis patients with or without *Helicobacter pylori* infection regarding iron deficiency anemia[21].

While on the other hand, a study carried out in the United States has revealed the association of *Helicobacter pylori* infection with iron deficiency both in the presence or absence of peptic ulcer disease[22]. It has been also reported that iron deficiency anemia in patients with asymptomatic gastritis was corrected successfully when they were given eradication therapy for *Helicobacter pylori*[23]*.* A study carried out in Turkish subjects also showed association of iron deficiency with *Helicobacter pylori* infection[24]. Another study conducted in Korean children had documented decreased serum ferritin levels in patients with *Helicobacter pylori* infection[25]. Low hemoglobin, ferritin, and B_{12} levels with *Helicobacter pylori* infection also have been reported in Pakistani population[26].

Our results did not reveal any effect of *H. pylori infection* on vitamin B12 levels but controversial picture is also present in literature about the vitamin B_{12} status in *Helicobacter pylori* infected cases. Similarly like us, no significant effect of *Helicobacter pylori* infection was reported on both vitamin B_{12} levels and serum ferritin levels[27]. *Helicobacter pylori* infection has not been suggested as the cause of B_{12} deficiency in alcoholic patients[28]. No relationship was observed between *Helicobacter pylori*, inflammatory changes, degree of gastritis and deficiency of vitamin B_{12} levels[29].The results of the study are important because it was carried out in a poor and developing country where poor socioecomic status, housing, sanitation, and water contamination are major predisposing factors as reported for *Helicobacter pylori* infection.

REFRENCES

1. Chan WY, Hui PK, Leung KM, Chow J, Kwok F, Ng CS. "Coccoid forms of *Helicobacter pylori* in the human stomach". *Am J Clin Pathol*. 1994; 102: 503–7.
2. Palmer KR, Penman ID, Paterson- Brown S. Alimentary Tract and Pancreatic Diseases. In: Boon NA, Colledge NR, Walker BR, Hunter JAA, eds. Davidson's Principles and Practice of Medicine. 20th ed. London. Elsevier. 2005: 849-934.
3. Kumar P, Clark M. Kumar and Clark's Clinical Medicine. 7th ed. London: WB Saunders. 2002:253-334.
4. Kuipers EJ, B MJ. Acid peptic disease: Epidemiology and Pathobiology. In: Goldman L, Ausiello D, eds. Cecil Medicine. 23rd ed. Philadelphia: Elsevier. 2007: 1009-13.
5. Blaser MJ, Atherton JC. *Helicobacter pylori* persistence: biology and disease.*J Clin Invest*. 2004;113:321–33.
6. Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S.Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. *Science*. 2003;300:1430–34.
7. Letley DP, Rhead JL, Twells RJ, Dove B, and Atherton JC. Determinants of non-toxicity in the gastric pathogen *Helicobacter pylori*. *J. Biol. Chem*. 278:26734–41.
8. Calam J. Peptic ulcer disease. In: Warrel DA, Cox TM, Firth JD, Benz Jr JD, eds. Oxford Textbook of Medicine. United Kingdom: Oxford University Press. 2010: 558-568.
9. Konturek SJ, Konturek PC, Pieniazek P, Bielanski W. Role of *Helicobacter pylori* infection in extragastroduodenal disorders: introductions remarks. *J PhysiolPharmacol.* 1999; 50:683–94.
10. Dierkes J, Ebertb M, Malfertheinerb P, Luleya C. *Helicobacter pylori* Infection, vitamin B_{12} and Homocysteine. Dig Dis. 2003; 21:237–44.
11. Kaptan K, Beyan C, Ural AU, Cetin T, Avcu F, Gülşen M, Finci R, Yalçín A. *Helicobacter pylori* – is it a novel causative agent in vitamin B_{12} deficiency. *Arch Intern Med 2000*; 160:1349–53.
12. Clyne M, Dolan B, Reeves EP. Bacterial factors that mediate colonization of the stomach and virulence of *Helicobacter pylori*.2007. *FEMS MicrobiolLett*; 268:135–43.

13. Choe YH, Oh YJ, Lee NG, Imoto I, Adachi Y, Toyoda N, Gabazza EC. Lactoferrin sequestration and its contribution to iron deficiency anemia in *Helicobacter pylori* infected gastric mucosa. *JGastroenterol Hepatol*. 2003; 18:980-5.
14. Salgueiro J, Zubillaga M, Goldman C, Barrado A, Martinez SM, Leonardi N, Boccio J.Is there a link between micronutrient malnutrition and Helicobacter pylori? *Aliment PharmacolTher*. 2004; 20:1029-34.
15. El-Omar EM, Oien K, El-Nujumi A, Gillen D, Wirz A, Dahill S, Williams C, Ardill JE, McColl KE. *Helicobacter pylori* infection and chronic gastric acid hyposecration. *Gastroenterology* 1997; 113:15-24.
16. Collect JA, Burt MJ, Frampton CM, Yeo KH, Champman TM, Buttimore RC, Cook HB, Chapman BA. Seroprevalance of *Helicobacter pylori* in the adult population of Christchurch: risk factors and relationship to dyspeptic symptoms and iron studies*. N Z Med J* 1999; 112:268–74.
17. Milman N, Rosenstock S, Anderson L, Jorgensen T, Bonnevie O. Serum ferritin, hemoglobin, and *Helicobacter pylori* infection: a seroepidemiologic survey comprising 2794 Danish adults. *Gastroenterology* 1998; 115:268–74.
18. Hacihanefioglu A, Edebali F, Celebi A, Karakaya T, Senturk O, Hulagu S. Improvement of complete blood count in patients with iron deficiency anemia and *Helicobacter pylori* infection after the eradication of *Helicobacter pylori*. *Hepatogastroenterology* 2004; 51:313–5.
19. Mahalanabis D, Islam MA, Shaikh S, Chakrabarty T, Kurpad AV, Mukherjee S, Khaled MA, Vermund SH. Hematological response to iron supplementation is reduced in children with asymptomatic *Helicobacter pylori* infection. *Br J Nutr* 2005; 94:969–75.
20. Lopez T, Quesada M, Almirall J, Sanfeliu I, Segura F, Calvet X. *Helicobacter pylori* does not contribute to iron deficiency in hemodialysis patients. *Nefrologia* 2006; 26:673–8.
21. Cardenas VM, Mulla ZD, Otiz M, Graham DY. Iron deficiency and *Helicobacter pylori* infection in the United States. *Am J Epidemiol*. 2006; 163: 127–34.
22. Annibale B, Marignani M, Monarca B. Reversal of iron deficiency anemia after *Helicobacter pylori* eradication in patients with asymptomatic gastritis. *Ann Intern Med* 1999; 131:668–72.
23. Suoglu OD, Gokee S, Saglam AT, Sokucu S, Saner G. Association of *Helicobacter pylori* with gastroduodenal disease, epidemiologic factors and iron deficiency anemia in Turkish children undergoing endoscopy, and impact on growth. *Pediatr Intern* 2007; 137: 119-24.
24. Seo JK, Ko JS, Choi KD. Serum ferritin and helicobacter pylori infection in children: a sero-epidemiological study in Korea. *J Gastroenterol Hepatol*. 2002; 17:754-7.
25. Zuberi BF, Afsar S, Qadeer R, Baloch I, Quraishy MS, Akhtar N. Hemoglobin, ferritin, vitamin B_{12} and Helicobacter pylori infection: a study in patients who underwent upper GI endoscopy at civil hospital Karachi. *J Coll Physicians Surg Pak*. 2007; 17:546-9.
26. Akcam M, Ozdem S, Yilmaz A, Gultekin M, Artan R. Serum ferritin, vitamin B_{12}, folate, and zinc levels in children infected with *H. Pylori*. *Dig Dis Sci* 2007; 52:405–10.
27. van Oijen MG, Laheij RJ, de Jong CA, Peters WH, Jansen JB. Vitamin B_{12} status and its association with Helicobacter pylori infection in alcohol dependent patients. *J Nutr Sci Vitaminol*. 2004; 50:305-8.
28. Rogers LM, Boy E, Miller JW, Green R, Rodriguez M, Chew F, Allen LH. Predictors of cobalamin deficiency in Guatemalan school children. Diet, Helicobacter pylori, or bacterial overgrowth? *J Pediatr Gastroenterol Nutr*. 2003; 36:27-36.
29. Serin E, Gumurdulu Y, Ozer B, Kayaselcuk F, Yilmaz U, Kocak R. Impact of *Helicobacter pylori* on the development of vitamin B_{12} deficiency in the absence of gastric atrophy. *Helicobacter* 2002; 7:33–41.