Original Paper

nephron Clinical Practice

Nephron Clin Pract 2004;96:c28–c32
DOI: 10.1159/000075569

Received: December 22, 2003
Accepted: September 30, 2003

Low Initial Vitamin B_{12} Levels in *Helicobacter pylori*-Positive Patients on Chronic Hemodialysis

H. Trimarchi M. Forrester J. Schropp H. Pereyra E.A. Freixas

Nephrology Section, Hospital Británico de Buenos Aires, Argentina

Key Words

Vitamin B_{12} · *Helicobacter pylori* · Hemodialysis · Cyanocobalamin · Folic acid · Homocysteine · Macrocytosis

Abstract

Background: *Helicobacter pylori* has been identified as a possible cause of vitamin B_{12} deficiency in the general population. We assessed any potential relationship between low cyanocobalamin serum levels and *Helicobacter pylori* status in hemodialysis patients and subsequently correlated these results with the existence of anemia (a common complication in hemodialysis patients), and macrocytosis. ***Methods:*** In 29 chronic hemodialysis patients, active *H. pylori* infection was diagnosed using two different methods regardless of digestive symptoms: by searching for bacterial antigens in stools and by the detection of urea breakdown through breath testing. If these results were non-coincident, gastroscopy was performed and antral biopsies obtained. Patients were subsequently divided into group A (*H. pylori*-positive, n = 8, 28%) and group B (*H. pylori*-negative, n = 21, 72%). The corresponding initial values of erythrocytic folic acid, vitamin B_{12} and homocysteine prior to the first hemodialysis session of each patient were retrospectively collected. ***Results:*** Vitamin B_{12} levels (normal 200–900 pg/ml) were significantly lower in group A compared to group B (225.4 ± 111.9 vs. 707.9 ± 258.3 pg/ml, $p < 0.011$). In group A, 5 patients (63%) had vitamin B_{12} deficiency (154 ± 24.6 pg/ml). Baseline hematocrits, erythrocyte folic acid and serum homocysteine levels were not different between the groups, but mean corpuscular volumes were significantly higher in group A compared to group B (109.7 ± 14.1 vs. 91.8 ± 8.8 fl, $p = 0.002$). ***Conclusions:*** *H. pylori*-positive chronic hemodialysis patients may present with lower vitamin B_{12} blood levels and macrocytosis. *H. pylori* infection should be suspected in this population when low or low-normal vitamin B_{12} levels or macrocytosis exist.

Introduction

Vitamin B_{12} (vitB12) deficiency is a known cause of megaloblastic anemia and polyneuropathy in the general population [1], two frequent complications encountered in the chronic renal failure community [2, 3]. As anemia in end-stage renal disease is primarily caused by decreased production of erythropoietin by the kidneys, virtually all patients who are started on dialysis are anemic if no treatment has been prescribed before [2]. With respect to neuropathy, subclinical manifestations can be detected

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Hernán Trimarchi
Servicio de Nefrología, Hospital Británico de Buenos Aires
Perdriel 74 (1280)
Buenos Aires (Argentina)
Tel. +54 114 309 6400, Fax +54 114 304 3393, E-Mail htrimarchi@hotmail.com

in over 50% of dialysis patients [3]. Abnormally low vitB12 blood levels have been estimated to affect 10–15% of people older than 60 years [4, 5] and reported in approximately 30–50% in hospitalized patients who have macrocytic anemia [6]. vitB12 deficiency can be due to an inability to release cobalamin from food, to autoimmune disorders in which antibodies are targeted against gastric parietal cells or intrinsic factor, or to ileal resections and subsequent vitamin malabsorption [7]. It has recently been proposed that *Helicobacter pylori* (HP), the most common gastric infection worldwide, appears to be a novel and causative agent of vitB12 deficiency in the general population [8]. We determined the prevalence of vitB12 deficiency and HP seropositivity in our hemodialysis (HD) unit and assessed the potential correlation between the bacterial infection and cobalamin depletion and any implications on anemia or macrocytosis.

Methods

H. pylori *Status*

HP infection was assessed using two non-invasive methods: an enzyme-linked immunoasssay (Premier-Platinum-HpSA; Meridian Diagnostics, Cincinnati, Ohio, USA) against HP antigens in patients' stools; and the urea breath test, which relies on bacterial hydrolysis of isotope-labeled carbon dioxide after oral administration of isotope-labeled urea (INER Hp ^{13}C-tester oral crystal; National Commission of Atomic Energy, Buenos Aires, Argentina). If these results were non-conclusive, a gastric endoscopy was performed and biopsies obtained.

Study Design and Patient Characteristics

According to these results, patients were divided into two groups: group A consisted of HP-positive patients (n = 8, 28%) and group B of HP-negative patients (n = 21, 72%). In the 29 chronic HD patients (table 1) included in this study, none had a recent past history of peptic ulcer disease or had been on proton pump inhibitors, H_2-blockers or drugs that could interfere with folic acid or vitB12 intestinal absorption in the recent past. All patients were Caucasian and had no history of hepatitis B, hepatitis C or human immunodeficiency virus infection or liver disease.

Blood Chemistries

Serum vitB12 (normal: 200–900 pg/ml) and erythrocytic folate levels (normal 200–700 ng/ml) were measured by a radioimmunoassay (Dualcount Slid-Phase, No-Boil Assay for Vitamin B_{12}/Folic Acid; Diagnostic Products Corp., Los Angeles, Calif., USA); homocysteine (normal: 10 ± 5 μmol/l) was determined by fluorescence polarization immunoassay. In addition, in group A, due to lower vitB12 titers, the presence of gastric parietal cell antibodies was determined (Biosystems SA, Costa Brava, Barcelona, Spain). Hematocrit levels and mean corpuscular volumes (MCV) were performed using an automatic counter. Macrocytosis was defined as a MCV greater than 96 fl.

Table 1. Patient characteristics

	Group A (n = 8)	Group B (n = 21)
Age, years	56.8 ± 13.1	62.4 ± 14.8
Female, %	63	59
Time on HD, months	18.9 ± 1.1	20.1 ± 2.1
Caucasian, %	100	100
Middle class, %	100	100
Diabetics, %	25	19

HD = Hemodialysis.

Analysis of HP Status and Blood Determinations

When HP infection was diagnosed, and assuming that HP is a chronic infection, blood determinations of homocysteine, folate and vitB12 (routinely withdrawn prior to the first hemodialysis session at our center) were retrospectively collected and matched with the HP status of each patient. These pre-dialysis determinations were considered as baseline, since these patients were not at the time receiving folate or vitB12 supplementation, but they would be receiving such prescription after their first hemodialysis session.

Hemodialysis Topics

Hemodialysis was performed using biocompatible polysulphone membranes (F80 Fresenius®, Hamburg, Germany) thrice a week using bicarbonate as a buffer. Each hemodialysis session lasted 3.5 ± 0.5 h and KT/V averaged 1.2 ± 0.2. The QB ranged between 300 and 400 ml/min and the QD was 500 ml/min. Erythropoietin was used subcutaneously at a dose of 70 U/kg/week to maintain a target hematocrit of 34% and intravenous iron saccharate was administered when appropriate to maintain a transferrin saturation index between 30 and 50%. No transfusions were prescribed during the study.

Statistics

Results are expressed as the mean ± SD. Kruskal-Wallis test (one-way ANOVA by ranks) was used for differences between groups. Wilcoxon signed ranks test was used to evaluate intragroup differences. $p < 0.05$ was considered significant.

Results

Results are depicted in tables 2 and 3. The prevalence of HP infection in our HD unit was 28%. In the total group of patients, mean vitB12 levels were 466.65 ± 185.1 pg/ml and MCV values 100.75 ± 11.45 fl. Patients with HP infection had significantly lower initial serum vitB12 levels when compared to HP-negative patients (225.4 ± 111.9 vs. 707.9 ± 258.3 pg/ml, $p < 0.011$) and higher MCV (109.7 ± 14.1 vs. 91.8 ± 8.8 fl, $p < 0.002$). In 2 patients from group A (25%) and in 3 patients from group B (14.3%), gastric endoscopies were performed

Table 2. Summarized laboratory results

	Group A	Group B
Initial hematocrit, %	28.1 ± 1.9	30.3 ± 1.2
Mean corpuscular volume, fl*	109.7 ± 14.1	91.8 ± 8.8
Serum creatinine, mg/dl	9.8 ± 2.3	9.1 ± 2.7
Initial eFA (normal: 200–700 ng/ml)	484 ± 122	307.8 ± 132.5
Initial vitB12 (normal: 200–900 pg/ml) **	225.4 ± 111.9	707.9 ± 258.3
Initial homocysteine (normal: 10 ± 5 μmol/l)	21.1 ± 6.3	24.2 ± 9.9

* $p < 0.002$; ** $p < 0.011$.
eFA = Erythrocytic folic acid.

Table 3. Group A patients' data

Patient	vitB12 pg/ml	Homcysteine μmol/l	MCV, fl	Serum creatinine, mg/dl
1	266	15	124	12.1
2	179	12	115	8.3
3	142	22	104	10.8
4	135	21	99	9.9
5	715	17	120	7.5
6	183	31	111	9.6
7	133	26	109	8.9
8	850	25	96	10.9

MCV = Mean corpuscular volume.

because the two diagnostic tests were negative. In group A, biopsies showed the presence of HP in the antral mucosa and a background of chronic gastritis, while in group B, biopsies were negative for HP. In the rest of the patients the results of the two non-invasive diagnostic methods were coincident. Moreover, in group A, 5 patients (63%) had vitB12 depletion (145 ± 24.6 pg/ml), albeit hematocrit in this subgroup of patients was not different from the rest. The frequency of vitB12 in our HD unit was 17.2%. In group A, only 1 patient (12.5%) had gastric parietal cell antibodies, but gastric biopsies were not consistent with atrophic gastritis or suggestive of pernicious anemia. Macrocytosis was present in 28% of patients, encountered in all patients from group A (100%) but in only 2 patients from group B (9.5%).

Discussion

Our results show that HP patients had lower baseline vitB12 serum levels, and that 63% of them had cobalamin deficiency. Although no correlation was found between vitB12 deficiency and anemia, macrocytosis was present in the HP-positive population, in agreement with a study by Kaptan et al. [8].

HP is a common human pathogen implicated in certain gastrointestinal diseases, such as chronic gastritis, gastric and duodenal ulcers, and has been recognized as a gastric carcinogen [9, 10]. Infection with HP is very common worldwide, occurring in 40–50% of the population in developed countries and in up to 90% of the population in developing countries [11–13]. In HD patients, the prevalence of HP infection depends on the diagnostic method employed, ranging between 56% when a serologic test was used [14] and 64% when the urea breath test was performed [15]. In our HD population, HP prevalence was 28% when the two methods and gastroscopy were used together. Despite the latest recommendations outlined by the American College of Gastroenterology that the search of HP infection in the general population is indicated only if treatment is intended in patients with active peptic ulcer disease, a past history of documented ulcer disease or gastric lymphoma [16], we believe that infection with HP should be investigated in HD regardless of a history of ulcer disease or related symptoms due to its relationship to vitB12 deficiency and its consequences. Our study performed on HD patients suggests that two methods should be undertaken in order to make the diagnosis of HP infection; if the results are non-coincident, a gastroscopy is a valid option.

HP has also been linked to many non-gastrointestinal tract conditions. However, the evidence in support of HP infection as a cause of the extraintestinal complications is not widely understood [17]. Some studies have found an association between HP infection and iron deficiency anemia, probably due to the acquisition of iron from the host and to an increased risk of chronic atrophic gastritis and peptic ulcer disease [17]. However, we have found that the hematocrit levels were not different between both groups, although macrocytosis was present in HP-infected patients. Curiously, in a recent investigation in non-HD patients, HP has been implicated as a novel cause of vitB12 deficiency [8], albeit no conclusive pathophysiologic relationship between cobalamin deficiency and HP exists. Macrocytosis, which can be caused by cyanocobalamin depletion, has been associated with HP infection both in that study as well as in ours.

In HP-positive patients, the effects of antibiotic therapy on serum vitB12 and MCV were regrettably not assessed due to the following reasons: 3 patients did not accept being started on antibiotics despite medical prescription, the circumstances being the lack of symptoms relating to HP infection and the large amount of medications they were already taking; 2 patients started treatment, but it was discontinued due to side effects. Only 3 patients completed treatment, which consisted of lanzoprazole 30 mg/twice a day, amoxicillin-clavulanic acid 2 g/day and clarithromycin 250 mg/twice a day for 14 days. This evidence would have been important to strengthen our hypothesis.

In HD patients, polyneuropathy and anemia are two frequent and important complications, with both morbidity and mortality implications. Although polyneuritis has not been evaluated in our study and hematocrit levels were not different between both groups, vitB12 low levels are a potential cause or an aggravating factor of both entities. vitB12 deficiency has been established as a cause of resistance to recombinant erythropoietin treatment in HD patients [2]. Macrocytosis could serve as a clue to investigate the presence of megaloblastosis, of which HP infection appears to be a cause.

In HD patients, macrocytosis has been reported in 38% of cases, folic acid deficiency being the main cause [18]; however, in our study, the prevalence was 30%, but erythrocyte folic acid levels were normal. In HD, macrocytosis can also occur in the setting of intravenous iron therapy in patients with co-existent folic acid deficiency [19], or can be induced directly by iron overload with a high reticulocyte count [20] and lacks specificity when used as a marker of a hematologic disorder [21]. In addition, in one study no correlation was found between folic acid or vitB12 levels and the mean corpuscular volume [18]. To our knowledge, no previous reports in dialysis patients have focused on whether HP could contribute to macrocytosis due to cyanocobalamin deficiency, a well-known cause of megaloblastosis in the general population. In our small and selected group of HD patients, HP infection appears to be linked to low blood levels of cyanocobalamin and macrocytosis, independent of folic acid levels.

With respect to homocysteine levels we found no statistical difference between both groups despite lower vitB12 levels in group A. This could be due to the fact that although homocysteine levels depend on folic acid, cyanocobalamin and pyridoxine blood status, folic acid appears to be the most important factor that inversely determines the degree of hyperhomocysteinemia [22]. In our patients, erythrocyte folic acid levels were normal in both groups (table 2). Moreover, we have found that intensive methylcobalamin (the methylated form of cobalamin that acts as a cofactor for methionine synthetase in the conversion of homocysteine to methionine) therapy alone is unable to correct or improve hyperhomcysteinemia when folic acid levels are low [22]. In addition, a different degree of renal failure between both groups could also determine different homocysteine levels, but serum creatine levels were not different (table 2).

Finally, there is little information available linking the possible pathophysiological association between HP infection and vitB12 deficiency. As shown by Kaptan et al. [8], the eradication of HP with antibiotic therapy in a substantial group of patients and the restoration of anemia and vitB12 levels is strongly suggestive of this gram-negative rod's role in cyanocobalamin deficiency. HP infection leads to a persistent state of inflammation, namely chronic gastritis [23], and HP-positive patients have circulating IgG autoantibodies against epitopes on specialized gastric mucosal cells [24]. Studies suggest that autoimmunity may play a role in the development of HP gastritis. There may be a relationship between intrinsic factor produced by gastric parietal cells and antibodies produced by the host against HP. These antibodies or the bacteria itself may ultimately affect the parietal cells, the synthesis and/or function of intrinsic factor, or the function of R proteins that bind cobalamin in the stomach, subsequently lowering vitB12 blood levels.

In conclusion, HP may lead to low cyanocobalamin levels and macrocytosis in chronic HD. In turn, macrocytosis could be used as an initial marker of low vitB12 levels due to HP infection. The present study comprises a small number of patients, and results must be interpreted with caution. Whether HP contributes to worsen polyneuropathy and resistance to erythropoietin therapy is still to be determined.

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