
Therapeutic Trial of Cobalamin in Patients with Normal Serum Cobalamin Levels and Predicted Cobalamin Deficiency

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ABSTRACT

Diagnosis of cobalamin deficiency can be difficult due to the variation in clinical presentation and lack of specificity of the laboratory findings. Although hypersegmentation and macrocytosis are important findings observed in cobalamin deficiency they are not sensitive and specific. Additionally, cobalamin assays in commercial laboratories, being not reliable, makes the diagnosis more difficult. The metabolite assays, such as the total homocysteine and methylmalonic acid are costly, which restricts their widespread routine use. Our aim was to find out problems in diagnosis of cobalamin deficiency in general practice, and establish a better and cost-effective decision strategy for the diagnosis of this common clinical entity. Fifty patients with the diagnosis of cobalamin deficiency were retrospectively evaluated with respect to diagnosis. Normal cobalamin levels were observed at presentation in eight (16%) patients. Reticulocyte crisis was observed in all patients on the seventh day of cobalamin replacement therapy and all hematological parameters returned to normal at the end of three months of treatment. In anemic patients with clinical and biochemical findings suggestive of megaloblastic anemia, even though serum cobalamin level is normal, a therapeutic trial of cobalamin is cost effective and prevents delay in diagnosis.

Key Words: Cobalamin, Deficiency, Anemia, Therapeutic trial, Methylmalonic acid.

ÖZET

Normal Serum Kobalamin Düzeyi Olan ve Kobalamin Eksikliği Düşünülen Hastalarda Tedavi Amaçlı Kobalamin Kullanımı

Değişken klinik prezentasyon ve spesifik laboratuvar bulgularının olmaması nedeniyle kobalamin eksikliğinin tanısı zor olabilir. Kobalamin eksikliğinde hipersegmentasyon ve makrositoz önemli bulgular olmasına rağmen sensitivite ve spesifiteleri düşüktür. Ayrıca ticari kitleler serum kobalamin düzeylerinin ölçümünün güvenilir olmaması tanıyı daha da zorlaştırabilir. Homosistein ve metilmalonik asit gibi metabolitlerin ölçümünün oldukça pahalı olması yaygın kullanımını engellemektedir. Bu çalışmadaki amaç, genel pratikte sık rastlanan bu klinik tabloda daha kolay ve daha ucuz bir tanı ve tedavi stratejisi uygulamaktır. Retrospektif olarak kobalamin eksikliği tanısı

konmuş 50 hasta değerlendirildi. Sekiz (%16) hastada hastaneye başvurduğu esnada kobalamin seviyeleri normaldi. Bu hastaların hepsinde kobalamin tedavisinin yedinci gününde retikülosit krizi gözlemlendi ve tedavinin üçüncü ayının sonunda bütün hematolojik parametreleri normale döndü. Klinik ve laboratuvar bulguları ile megaloblastik anemi düşünülen hastalarda serum kobalamin seviyeleri normal olsa bile, kobalamin ile deneme tedavisi hem ekonomiktir hem de tanının gecikmesini önler.

Anahtar Kelimeler: Kobalamin, Kobalamin yetmezliği, Anemi, Terapötik deneme, Metilmalonik asit.

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INTRODUCTION

Cobalamin deficiency is a commonly encountered problem in general practice and prevalences of 5-15% in elderly populations have been reported^[1-3]. Diagnosis of cobalamin deficiency is based on medical history and the finding of megaloblastic anemia combined with low serum cobalamin concentration. Since the development of cobalamin deficiency is slow, the symptoms may be vague. Symptoms and signs of vitamin B12 deficiency are variable and include loss of sensation of vibration and position, ataxia, loss of memory, decreased reflexes, positive Babinski's sign, urinary and/or fecal incontinence, impotence, abnormal taste and odor sensation, visual problems and optic atrophy, depression, psychosis, acute confusional state, hallucinations, panic attacks, personality changes and tendency to suicide^[4-7]. Neurological or psychiatric symptoms and signs may be observed without anemia or macrocytosis, and may lead to erroneous referrals^[8].

A variety of laboratory findings can be observed in cobalamin deficiency, including anemia, reticulocytopenia, macrocytosis, neutropenia, thrombocytopenia, hypersegmentation of the neutrophils, elevated lactate dehydrogenase, and increased unconjugated bilirubin in the serum^[9]. The variation in clinical and laboratory findings may lead to delayed diagnosis, which may in turn cause increased morbidity. Furthermore, the cobalamin assays in commercial laboratories, being not reliable, makes the diagnosis more difficult^[10,11]. Tests, indicating functional cobalamin status, including plasma total homocysteine and serum methylmalonic acid (MMA) may be used for diagnosis, but the main limitation to widespread use of these metabolite assays as the primary test is their high cost^[12]. The costs of MMA assays are about 10-fold higher than that of serum cobalamin^[12].

Our aim was to find out problems in diagnosis of cobalamin deficiency in general practice, and establish a better and cost-effective decision strategy for the diagnosis of this common clinical entity.

PATIENTS and METHODS

Patients diagnosed as cobalamin deficiency in the hematology out-patient clinics of Marmara University Hospital in İstanbul and Karadeniz Technical University in Trabzon, between February 1997 and June 2000, were recruited to the study. They were retrospectively evaluated for age, sex, date of the first symptoms, reason for referral, serum cobalamin concentrations, concomitant illnesses, iron and folic acid levels, antiparietal cell antibody positivity, reticulocyte count prior to and seven days following cobalamin replacement, peripheral blood smear assessment and complete blood counts prior to and three months following cobalamin replacement. Neutrophil hypersegmentation was documented by observing one or both of the following criterias:

1. Over 5% of the neutrophils having five segments,
2. The presence of at least one neutrophil containing six or more lobes.

Decision for cobalamin replacement was given according to the clinical, hematological, biochemical and bone marrow aspiration smear assessment (if needed). Cobalamin (Dodex, Deva) was administered with a dosage of 1 mg/day intramuscularly for five consecutive days, followed by five weekly injections, if reticulocyte crisis was observed on seventh day, and once a month thereafter. Cobalamin deficiency was confirmed with complete response to therapeutic trial. Oral iron and folic acid was administered to patients with concurrent low ferritin and folic acid levels, respectively.

Cobalamin Assay

Microparticle enzyme immunoassay (MEIA) method, Abbot, AxSYM (Cat No: 3C79), was employed for serum cobalamin assay. Six point standard calibration procedure was used.

RESULTS

During the 30 month period 50 patients (26 female, 24 male) were diagnosed to have cobalamin deficiency. Patients' characteristics are shown in Table 1. Median age of the patients was 54 ± 17 years. Forty-four (88%) patients presented with symptoms of anemia, four (8%) with neurologic complaints and two (4%) with glossitis and stomatitis. Hematological evaluation revealed anemia in 48 (96%), thrombocytopenia in 20 (40%), neutropenia in 13 (26%) patients. Two patients without anemia were referred to haematology clinic for leukopenia. Mean cell volume was high, normal or low in 36 (72%), nine (18%) and five (10%) patients respectively. Hypersegmentation of neutrophils was observed in 41 (82%) patients. Serum ferritin and folic acid levels were low in four (8%) and two (4%) patients respectively. Serum cobalamin levels were found to be low in 42 (84%), and normal in eight (16%) patients. Anti-parietal cell antibody titers were positive in 27 (54%) subjects. Four (8%) patients had a past history of sub-total gastrectomy. Of all the patients bone marrow aspiration smear examination was needed for only two patients who were in normal serum cobalamin group, and findings were hypercellular bone marrow with erythroid hyperplasia and megaloblastic

Table 1. Patients' characteristics (n= 50)

Median age	54 \pm 17
Presenting symptoms	
Anemia	44 (88%)
Neurologic symptoms	4 (8%)
Glossitis and stomatitis	2 (4%)
Laboratory features	
Anemia	48 (96%)
Thrombocytopenia	20 (40%)
Neutropenia	13 (26%)
Hypersegmentation	41 (82%)
APCA (+)	27 (54%)

changes in both of them. Reticulocyte response was observed in all patients on the seventh day of cobalamin replacement therapy, except two patients with normal hemoglobin at presentation. These two patients were referred with leukopenia and had neurologic symptoms which improved with cobalamin treatment. Hematological parameters of all patients returned to normal at the end of three months of cobalamin replacement therapy, except one female patient whose hemoglobin increased from 8.2 g/dL to 11.4 g/dL and MCV decreased from 76 fl to 55 fl. She was then diagnosed as β -thalassemia trait by hemoglobin electrophoresis.

DISCUSSION

Clinical and laboratory abnormalities caused by cobalamin deficiency are variable and nonspecific. Macrocytosis (MCV > 96 fl) is an important parameter of cobalamin deficiency. The MCV tends to increase before the hemoglobin level decreases significantly^[13]. However, even in the presence of biochemical evidence of vitamin B12 deficiency, the MCV may remain normal or low, especially in subjects with accompanying iron deficiency or thalassemia trait^[14-17]. Low MCV levels in five patients and normal MCV levels in nine patients were observed in this study. Four of the patients with low MCV had iron deficiency and one had β -thalassemia trait. The MCV also lacks specificity for the diagnosis of cobalamin deficiency. In a study, in patients with MCV greater than 115 fl, less than 50% of patients had subnormal serum cobalamin levels, and only an MCV higher than 130 fl reliably predicted a low serum cobalamin level^[18].

Hypersegmentation of the neutrophils observed in peripheral blood smear is another indicator of cobalamin deficiency, but it also lacks specificity and sensitivity. Metz et al showed neutrophil hypersegmentation in two thirds of the patients with low vitamin B12 levels and in 4% of the normal controls^[19]. Hypersegmentation was observed in 82% of the patients in this study.

Measurement of serum cobalamin concentration has been the cornerstone for the diagnosis of cobalamin deficiency. However, the diagnostic efficiency of serum cobalamin is low^[20]. This can partly be explained by the fact that the major fraction of cobalamin in serum is bound to haptocorrin, which is not available

for uptake in most cells^[20]. Only the subcomponent of vitamin B12 that is bound to transcobalamin II is the biologically active form of the vitamin^[21]. Additionally, serum cobalamin concentrations are directly altered by the binding proteins^[22]. Falsely increased values can be caused by myeloproliferative diseases^[21]. Falsely low levels can be seen with folate deficiency, pregnancy and transcobalamin deficiency^[21]. Intestinal bacterial overgrowth may cause falsely normal serum cobalamin levels because of the production of biologically inactive cobalamin analogues by the bacteria^[23]. Therefore, total serum cobalamin is a relatively poor indicator of bioavailable cobalamin.

Cobalamin deficiency in tissues, especially in brain, is associated with neuropsychiatric symptoms and signs. Determination of cobalamin deficiency in blood may fail to reflect the cobalamin status in tissues. Therefore, tests for other cobalamin markers have been developed, such as the total homocysteine and MMA^[12]. Serum MMA has attracted growing interest, since it is considered to reflect the functional status of cobalamin in the tissue. Norman et al used elevated urinary MMA levels as evidence of cobalamin deficiency, and follow up of 35 subjects with elevated urinary MMA levels in this study showed that 18 patients had low serum total cobalamin, 12 had low-normal cobalamin, and five had normal serum total cobalamin level^[24]. Holleland et al reported increased MMA concentrations in 12.7% of the subjects with serum cobalamin levels between 190-299 pmol/L^[20]. However, most of the metabolite assays are costly and this prevents their widespread routine use^[20]. Therapeutic trial in anemic patients is easy to evaluate since reticulocyte count rises in two to three days, with a peak in five-eight days^[25]. Thus, in clinical practice, if the patient's medical history, other laboratory tests or bone marrow aspiration smear are suggestive of megaloblastic anemia, a therapeutic trial may be preferred to the metabolite assays above.

In the present study, eight (16%) among 50 of the patients with cobalamin deficiency had normal cobalamin levels. Hematological parameters of these patients prior to and following cobalamin replacement therapy are illustrated in Table 2. Reticulocyte crisis was observed following cobalamin replacement therapy on the seventh day, and all hematological parameters returned to normal after three months in all these patients.

This study showed that in general practice normal

serum cobalamin level is used to rule out cobalamin deficiency, which causes delay in diagnosis and unnecessary referrals. In conclusion, in anemic patients with clinical, biochemical and bone marrow aspiration findings suggestive of megaloblastic anemia, even though serum cobalamin level are normal, a therapeutic trial of cobalamin is cost effective and prevents delay in diagnosis.

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Table 2. Blood counts prior to and following vitamin B12 replacement for patients with normal serum vitamin B12 concentrations

Patient	VitB12 (pg/mL)	Prior to vitamin B12 replacement				Following vitamin B12 replacement			
		Hb (g/dL)	MCV (fl)	WBC (/µL)	Plt (/µL)	Hb (g/dL)	MCV (fl)	WBC (/µL)	Plt (/µL)
1	337	8.5	61.5	4.300	126.000	15.0	90.7	5.400	155.000
2	299	5.1	114.7	5.200	98.000	13.1	80.5	6.000	204.000
3	212	5.2	117.0	3.200	62.000	15.1	92.8	12.400	302.000
4	487	6.6	112.0	3.600	115.000	13.3	82.0	7.900	265.000
5	355	9.4	88.2	1.600	50.000	13.0	80.5	7.700	176.000
6	400	7.0	125.0	3.100	214.000	12.9	89.1	6.900	189.000
7	425	5.3	128.4	2.900	72.000	14.2	88.4	7.900	210.000
8	320	4.5	122.0	1.700	112.000	13.6	90.1	4.000	230.000
Median	346 ± 84	5.9 ± 1.7	115 ± 22	3.150 ± 1.211	105.000 ± 51.000	13.0 ± 0.9	88 ± 4	7.300 ± 2.485	207.000 ± 48.000

Hb: Hemoglobin, MCV: Mean cell volume, WBC: White blood cell count, Plt: Platelet.

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