Vitamin B12 Deficiency with the Absence of Anemia in Young and Middle-Aged Population

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ABSTRACT

Vitamin B12 deficiency with macrocytic anemia is a well-known clinical entity. However the clinical importance of the vitamin B12 insufficiency without any hematological abnormality is underestimated in the adult patient population. The aim of this study is to assess frequency of vitamin B12 deficiency with normal complete blood counts (CBC) in adult subjects. Within the 1-year long period, the adult patients among the ones that had applied to Hacettepe University Hospitals were included in the study, in whom the vitamin B12 deficiency was detected. The patients, whose vitamin B12 values were <200 pg/ml were covered in the study. To identify the ages, genders, complete blood cell count values, the parameters of anemia, homocysteine values, were scanned and assessed. In sum, 4077 patients, in whom vitamin B12 deficiency had been detected were included in our study. Later, the patients were separated into two sub-groups, young and middle-aged patients, whose ages were between 17 and 49 (n= 2186). 71.3% of the 2186 young and mid-aged patients consisted of women while 28,7% consisted of men. The rest of the patients had vitamin B12 deficiency without any hematological abnormalities. Homocysteine level, which was recommended as a verification testing method was monitored in 14% of the patients. Significantly low levels of vitamin B12 were detected in the adult population without anemia, in study. The detection of vitamin B12 deficiency with normal CBC requires early intervention for both clinical diagnostic and/or therapeutic approaches. The verification tests required for final diagnosis.

Keywords: Vitamin B12, Deficiency, Anemia, Adults

ÖZET

Genç ve Orta Yaş Popülasyonda Aneminin Eşlik Etmediği Vitamin B12 Eksikliği

Vitamin B12 eksikliğine bağlı makrositik anemi iyi bilinen bir klinik durumdur. Bununla birlikte erişkin popülasyonda hematolojik anormallik olmadan görülen vitamin B12 eksikliği önemsiz görülebilmektedir. Bu çalışmanın amacı tam kan sayımı (CBC) normal olan hastalarda vitamin B12 düşüklüğünün sıklığının değerlendirilmesidir. Araştırmaya 1 yıllık periyotta Hacettepe Üniversitesi Hastaneleri' ne başvuran erişkin hastalar arasından vitamin B12 düşüklüğü tespit edilen erişkin hastalar dahil edildi. Vitamin B12 değeri <200 pg/ml olan hastalar alındı. Vitamin B12 düşüklüğü tespit edilen hastaların yaşları, cinsiyetleri, tam kan sayımı değerleri, anemi parametreleri, homosistein değerleri, endoskopi bilgileri retrospektif olarak taranarak değerlendirilmesi yapıldı. Çalışmamıza toplam 4077 vitamin B12 düşüklüğü tespit edilen hasta alındı. Daha sonra hastalar 17-49 yaş arası genç ve orta yaş olarak alt gruba ayrıldı (n= 2186). 2186 erişkin hastanın %71.3'ünü kadın %28.7'sini erkekler oluşturuyordu. Vitamin B12 eksikliği bulunan hastalarda hematolojik anormallik yoktu. Doğrulama testi olarak önerilen homosistein düzeyi ise %14 hastada bakılmıştı. Çalışmada anemi olmadan vitamin B12 düşüklüğünün çok belirgin olduğunu gördük. CBC normal vitamin B12 düşüklüğü tespit edilen hastaların erken dönemde tanı ve tedavi yaklaşımı için değerlendirilmeleri gerekmektedir. Kesin tanı için de doğrulama testleri yapılmalıdır.

Anahtar Kelimeler: Vitamin B12, Eksiklik, Anemi, Erişkinler

INTRODUCTION

Vitamin B12 is a water-soluble and essential vitamin which must be obtained from a dietary source.[1] Severe vitamin B12 deficiency causes megaloblastic anemia, neuropathy and myelopathy. Elderly population is at great risk for the development of vitamin B12 deficiency.[2] Vitamin B12 measurement is usually assessed in patients with macrocytic anemia. But, it should be remembered that vitamin B12 deficiency may lead to systemic symptoms or especially progressive neurological problems before the development of anemia. More importantly these problems can be irreversible. Vitamin B12 deficiency with normal blood counts is usually under estimated in clinical practice, only routinely assessed in geriatric population. The frequency of this problem is not known in young and middle-aged population. The aim of this study is to determine the frequency of vitamin B12 deficiency in young and middle-aged population and to detect the presence of anemia accompanying vitamin B12 deficiency by evaluating the other laboratory parameters in adult patients with low B12 levels. The tests required fo the confirmation of vitamin B12 deficiency and etiology in patients with low vitamin B12 levels are also evaluated. The vitamin B12 deficiency related complications can be prevented by increasing awareness and early diagnosis, confirmation of diagnosis, performing sufficient etiologic tests and determining appropriate treatment.[3,4]

PATIENTS AND METHODS

The adult patients who applied to Hacettepe University Hospitals in 1-year period and who had low serum vitamin B12 levels were included in our study. Normal serum vitamin B12 value, which is internationally accepted and used in biochemistry laboratories in our hospital, is 200-790 pg/ml. The patients whose vitamin B12 levels are <200 pg/ml and who are older than 17 years are included in the study. Patients who were admitted to inpatient service were excluded because of the reasons such as the presence of multiple comorbidity and drug treatments which may affect the vitamin B12 levels. The patients whose complete blood count results were not obtained were also excluded from the study, although they had low serum vitamin B12 levels. Patients with low vitamin B12 levels were retrospectively evaluated by considering age and gender, and screening complete blood count values, serum iron, iron-binding capacity, fer-

ritin, homocysteine values, presence of antiparietal antibody, celiac antibodies and endoscopic results.

Ethics committee approval was obtained prior to the study initiation.

Patients were divided into groups according to their ages. 17-29 year-old patients were grouped as young, 30-49 year-old as middle-aged and >65 as elderly. Severity of vitamin B12 deficiency was classified as severe (<100 pg/ml), moderate (100-149 pg/ml) and mild (150-200 pg/ml). 17-49 year-old patients were defined as young-to-middle-aged adult group.

Anemia-related parameters of adult patients were examined in detail. Hemoglobin levels of patients were measured to determine the presence of anemia. Male patients with hemoglobin level <13.6 g/dl and female patients with hemoglobin level <12 g/dl were defined as anemic. Moreover, the presence of iron deficiency anemia accompanying vitamin B12 deficiency was assessed. Patients with ferritin value <12 ng/ml or transferrin saturation <15% were included in iron deficient group.

The results of antiparietal antibody tests, homocysteine levels and celiac antibodies was recorded if screened in vitamin B12 deficient patients. Normal homocysteine level was defined as 0-12 mmol/L for patients older than 12 years. Endoscopic results, if present, of the adult patients were also examined.

Statistical Analysis

Statistical analysis of the data obtained in this study was performed by using Statistical Package for the Social Sciences (SPSS) 15.0 software program. Data was summed as mean±standard deviation and median. One-way analysis of variance was used to compare measurements from more than two groups while Mann-Whitney U Test was used to compare two groups. Interparameters relationship was evaluated by using Spearman's correlation coefficient. Chi-Square test was used when appropriate. $P<0.05$ value was accepted as statistically significant.

RESULTS

In order to detect the patients with vitamin B12 deficiency, vitamin B12 values which were measured in adults patients in 1-year period were analyzed in collaboration with biochemistry department. It was found that 40.782 vitamin B12 measurements were performed in adult patients in 1-year period. It was
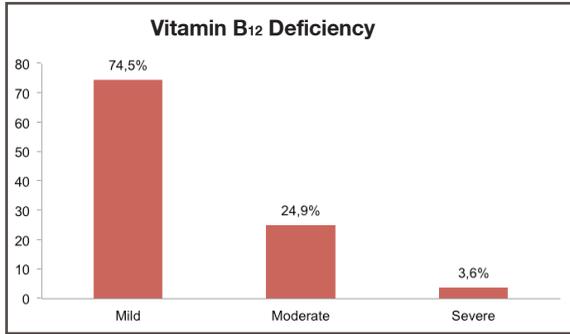
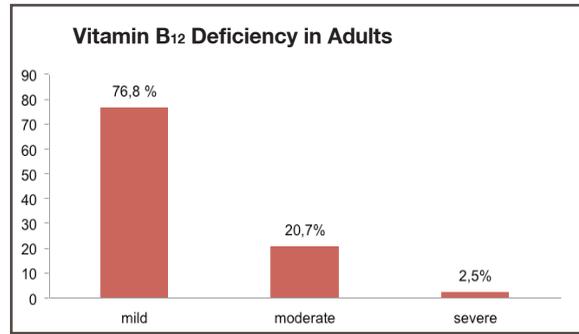**Figure 1.** Classification of vitamin B12 deficiency

Figure 2. The severity of vitamin B12 deficiency in adults

remarkable that vitamin B12 measurements were one and a half times more frequent than the measurements requested for the other anemia markers. As a result, 4077 patients were found to have vitamin B12 level lower than 200 pmol / L.

63.8% (n= 2600) were female and 36.2% (n= 1477) were male of 4077 patients, including all age groups. When severity of vitamin B12 deficiency was evaluated in all age groups, great majority was found to be in mild deficiency group (Figure 1).

Young and middle-aged patients group was consisted of 2186 patients. These patients similarly consisted of 71.3% (n= 1557) female and 28.7% (n= 629) male patients. When they were classified according to the severity of vitamin B12 deficiency, mild deficiency group was the majority as well (Figure 2).

Vitamin B12 deficiency levels according to the gender was also similar (Figure 3).

When rate of anemia was measured according to hemoglobin levels of adult patients, 21.5 % (n= 465) of 2156 patients were found to have anemia. Moreover, 16% (n= 101) of male patients and 23.3% (n= 364) of female patients had anemia.
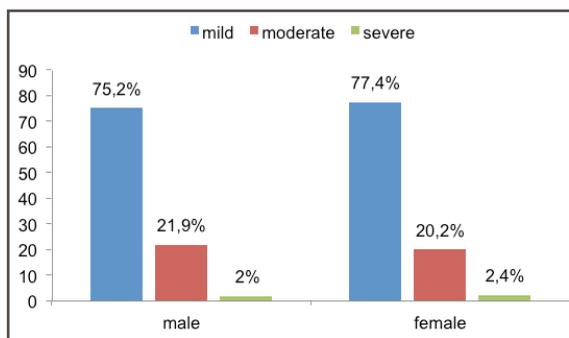
The patients with iron deficiency were detected by screening iron parameters of patients. It was found that iron parameters of 1039 patients (48%) were measured and 69% (n=715) of them had iron deficiency anemia (IDA) (Figure 4)

The patients, whose iron parameters were obtained, were analyzed according to the gender. While 8.8% (n= 63) of the patients with iron deficiency were male, 91.2% (n= 652) of them were female. The most common concomitant anemia was IDA.

When the rate of IDA was analyzed according to the age groups, it was found that 41% of patients with IDA were young patients and 59% of them were middle-aged patients. The difference was statistically significant (p< 0.005). We concluded that frequency of IDA increases with age.

Vitamin B12 deficient patients were analyzed according to gender considering their MCV values. The mean MCV value was found to be 87.7 fl for male and 87.0 fl for female patients. Although the difference between these values were statistically significant, they were not clinically significant.
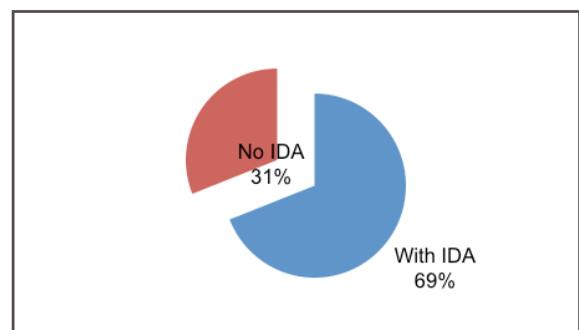

Figure 3. Severity of vitamin B12 deficiency according to gender

Figure 4. Iron deficiency anemia accompanying vitamin B12 deficiency (for the patients whose iron parameters were measured, n= 1039).

According to the confirmation tests, it was found that homocysteine levels were measured in only 12% (n= 260) of 2186 patients. Frequency of homocysteine measurements were also lower such as the other markers. In 36% of 260 patients, whose homocysteine levels were measured, homocysteine level was found to be high.

Frequency of antiparietal antibody measurements to assess the presence of pernicious anemia was also analyzed. It was found that APA were measured in 8% (n= 183) of patients. 21% of these patients were APA positive.

It was obtained that 8% (n= 179) of 2186 patients underwent endoscopy. Moreover, 10.1% (n= 18) of 179 patients had atrophic gastritis.

As an etiologic test, celiac antibodies were measured in 7% (n= 154) of 2186 patients and only 0.7% (n= 14) of these patients were found to be positive.

DISCUSSION

In our study, it is shown that anemia is present only in 21.5% of the patients with low serum vitamin B_{12} levels. In rest of the patients complete blood counts were normal. The majority of vitamin B_{12} deficient patients was female in young-to-middle-aged patient group. IDA accompanying B_{12} deficiency was also frequently observed in female patients.

Normal range of serum vitamin B_{12} level is 200-790 pg/ml. It has a broad range of reference. Its normal value, however, is not precisely known. Serum vitamin B_{12} level used for diagnosis has a limited specificity and sensitivity below 400 pg/ml.[5] Therefore, the values in the range of 200-400 pg/ml cannot exclude the presence of vitamin B_{12} deficiency. Clinical signs of vitamin B_{12} deficiency may be observed even in the normal reference ranges.[6] For this reason, additional tests such as measurements of methylmalonic acid (MMA), holotranscobalamin (HoloTC) and homocysteine should be performed for the vitamin B_{12} levels below 400 pg/ml. In this study, we set the reference range of vitamin B_{12} that biochemistry laboratory of our hospital used. We accepted the serum vitamin B_{12} value <200 pg/ml as B_{12} deficiency. It was found that confirmation tests for vitamin B_{12} deficiency were not frequently performed as much as vitamin B_{12} tests. Homocysteine levels were measured only in 12% of patients.

Additional tests such as upper endoscopy, H. pylori, antiparietal antibody and celiac antibody measurement tests regarding etiology should be performed for the patients who had low levels of vitamin B_{12} or who were diagnosed with vitamin B_{12} deficiency. In our study, we found that only 8% of patients with low vitamin B_{12} levels underwent upper endoscopy. We also observed that celiac antibodies were measured in 8% of patients. We concluded that etiologic tests are also less frequently performed, which is a similar result as confirmation tests.

The world-wide prevalence of vitamin B_{12} deficiency is not certainly known. In addition, it is known that its incidence increases with age. In a study, it was shown that 15% of patients older than 65 years has vitamin B_{12} deficiency. It has been mentioned that the prevalence of B_{12} deficiency occurring in adults has an average of 5-20%.[7,8] In a surveillance study which was performed in United States between 1999-2002, vitamin B_{12} deficiency was found to be 3% in 20-39 years of age, 4% in 40-59 years of age and 6% in >70 years of age when basal level of vitamin B_{12} was accepted as 148 pmol/L. When basal vitamin B_{12} value was accepted as 221 pmol/L, the prevalence of deficiency was 14-16% in 20-59 years of age and 20% in >60 years of age.[9,10] There is no surveillance study regarding the prevalence of vitamin B_{12} deficiency in Turkey. The number of studies about vitamin B_{12} levels and related factors are limited. In a cross-sectional study which was performed in Ankara, the rate of vitamin B_{12} levels <250 pg/mL, <200 pg/mL and <100 pg/mL were reported to be 67.4%, 46.8% and 6.5% (respectively) in patients underwent upper gastrointestinal endoscopy for dyspeptic complaints.[11] In a study performed in one of the Turkey's city Afyonkarahisar with 1100 participants between 41-64 years of age, it was found that vitamin B_{12} average was 202.95 pmol/L and serum vitamin B_{12} level was below 140 pmol/L in 29.7% of the population.[12] Although our study is not a surveillance study, vitamin B_{12} levels were found to be low in 4077 of 40.000 patients whose vitamin B_{12} level was measured. Almost 10% of the patients had low vitamin B_{12} levels. The patients whose vitamin B_{12} levels were below reference value was 5-6% of young adults, which is consistent with the current literature. It is obvious that screening of those patients for vitamin B_{12} deficiency with this frequency would not be cost-efficient. However, particular risk groups were determined. B_{12} levels should be routinely measured every 2-3

years and additional MMA, HoloTC, homocysteine tests should be performed, if required, in these risk groups.[13,14] Risk groups are specified as patients with unexplained anemia, unexplained neuropsychiatric disorder, the patients with diarrhea, anorexia, stomatitis symptoms, elderly patients, vegetarians, the patients with gastrointestinal diseases such as H. pylori infection, gastric resection and Crohn's disease.

In our study, high rate of B12 deficiency without hematologic changes such as anemia, macrocytosis was observed. Since it was not confirmed if this deficiency is directly related to low measurements of serum vitamin B12 level, it is difficult to interpret this inconsistency with hematologic data. The facts that it was unknown if B12 deficiency caused any clinical complaints or symptoms in patients and if patients had any other additional tests in another hospital, were limitations of our retrospective study.

B12 deficiency is a reversible disease with an early diagnosis and treatment. Therefore, it is important to be aware of this fact not only in elderly patients but also in young and middle-aged patients. It is also important to perform more confirmation tests and more etiologic screening to diagnose vitamin B12 deficiency anemia more accurately, and to provide cause-related treatment. It's also important to assess high risk patients for the development of vitamin B12 deficiency who might be candidate for frequent measurement and prophylactic replacement. Further prospective studies are needed to determine frequency vitamin B12 deficiency in young and middle aged adults and whether vitamin B12 deficiency leads to vitamin B12 deficiency anemia or not.

Significantly low levels of vitamin B12 were detected in the adult population without anemia, in the present study. The detection of vitamin B12 deficiency with normal CBC requires early intervention for both clinical diagnostic and/or therapeutic approaches. Because vitamin B12 deficiency may lead progressive health problems. The verification tests required for final diagnosis, should be applied accurately.

REFERENCES

1. Baik HW, Russell RM. Vitamin B12 deficiency in the elderly. Annu Rev Nutr 19: 357-377, 1999.

2. Carmel R. Megaloblastic anemias. Curr Opin Hematol 1: 107-112, 1994.

3. Maralcan M, Ellidokuz E. Vitamin B12 Eksikligi. Güncel Gastroenteroloji 8: 199-204, 2004.

4. Oh RC, Brown DL. Vitamin B12 Deficiency. AAFP 67: 979-986, 2003.

5. Herrmann W, Schorr H, Obeid R, Geisel J.Vitamin B12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. Am J Clin Nutr 78: 131-136, 2003.

6. Lesho EP, Hyder A. Prevalence of subtle cobalamin deficiency. Arch Intern Med 159: 407, 1999.

7. Wolters M, Ströhle A, Hahn A. Cobalamin: A Critical Vitamin in The Elderly. Prev Med 39: 1256-1266, 2004.

8. Danki D, Telci S, Dilbaz N, Okay IT. B12 vitamin eksikligine bagli psikotik bozukluk. Klinik Psikofarmakoloji Bülteni 16: 109-113, 2006.

9. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999-2000. Am J Clin Nutr 82: 442-450, 2005.

10. Pfeiffer CM, Johnson CL, Jain RB, et al. Trends in blood folate and vitamin B12 concentrations in the United States, 1988–2004. Am J Clin Nutr 86: 718-727, 2007.

11. Tangün Y. Hematoloji Birinci Basamak Kursu Egitim Kitabi. Izmir: Türk Hematoloji Dernegi 2000; 37-39, 2000.

12. Koken K, Toprak D, Dogan N. Prevalence of cobalamin deficiency in Afyonkarahisar elderly population. Clin Biochem 42: 4-5, 2009.

13. Herrmann W, Obeid R, Schorr H, Geisel J. The usefulness of holotranscobalamin in predicting vitamin B12 status in different clinical settings. Curr Drug Metab 6: 47-53, 2005.

14. Obeid R, Schorr H, Eckert R, Herrmann W. Vitamin B12 status in the elderly as judged by available biochemical markers. Clin Chem 50: 238-241, 2004.

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