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VITAMIN B12 AND FOLIC ACID LEVELS AFTER IRON THERAPY IN IRON-DEFICIENCY ANEMIA

DEMİR EKSİKLİĞİ ANEMİSİNDE DEMİR TEDAVİSİ SONRASI B12 VİTAMİNİ VE FOLİK ASİT SEVİYELERİ

Didar Yanardag Acik [1], Bilal Aygun [1]

1 Department of Internal Medicine and Haematology, Adana City Education and Research Hospital, Adana, Turkey

Sorumlu Yazar/Corresponding Author:Didar Yanardağ Açık E-mail: didaryanardag@gmail.com
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0000-0001-7282-0188, 0000-0001-7368-8123

Abstract
Objective: In this study, our intent was to determine whether serum levels of B12 and folic acid in patients diagnosed with iron deficiency anemia undergo change in response to iron supplements.
Methods: In this retrospective study, 202 female patients with iron deficiency anemia and only iron treatment were included. Complete blood count, vitamin B12, ferritin, folate levels of patients at the time of initial presentation and 4-8 weeks after treatment were examined.
Results: Overall, significant increases were detected in mean hemoglobin, mean hematocrit, mean corpuscular volume, median white blood cell and median serum ferritin levels in all patients treated with iron therapy (both oral and intravenous); significant decreases were observed in post-treatment median platelet, median folate, and median B12 levels in this patient cohort.
Conclusions: Erythropoiesis slows in the deficiency of any of the critical factors that promote erythropoiesis, serum vitamin B12, folate, and iron. We can say that the serum levels of these vitamins decrease with the use of vitamin B12 and folate after increased erythropoiesis with iron treatment.

Keywords: Vitamin B12, folic acid, Iron deficiency anemia, Iron therapy

Öz
Giriş: Bu çalışmada amacımız, demir eksikliği anemisi teşhisi konulan hastalarda serum B12 ve folik asit düzeylerinin demir takviyesine yanıt olarak değişip değişmediğini belirlemekti.
Materyal metot: Retrospektif yapılan bu çalışmaya demir eksikliği anemisi olan ve sadece demir tedavisi alan 202 kadın hasta dahil edildi. Hastaların ilk başvuru anında ve tedaviden 4-8 hafta sonra tam kan sayımı, B12 vitamini, ferritin, folat seviyeleri incelendi.
Bulgular: Genel olarak, demir tedavisi ile tedavi edilen tüm hastalarda (hem oral hem de intravenöz) ortalama hemoglobin, ortalama hematokrit, ortalama korpüsküler hacim, medyan beyaz kan hücresi ve medyan serum ferritin seviyelerinde önemli artışlar tespit edildi; bu hasta kohortunda tedavi sonrası medyan trombosit, medyan folat ve medyan B12 seviyelerinde önemli düşüşler gözlenmiştir.
Sonuç: Eritropoezi teşvik eden kritik faktörler olan serum vitamin B12, folat ve demirin herhangi birinin eksikliğinde eritropoez yavaşlar. Demir tedavisi ile artan eritropoez sonrası vitamin B12 ve folatın da kullanılmasıyla birlikte bu vitaminlerin serum seviyelerinin düştüğünü söyleyebiliriz.

Anahtar kelimeler: Vitamin B12, folik asit, demir eksikliği anemisi, demir tedavisi

Introduction

Iron deficiency and iron deficiency anemia (IDA) are diagnosed worldwide; this finding is particularly common among women of reproductive age[1]. Folate, vitamin B12 (B12) and iron are critical for normal erythropoiesis; erythroblasts specifically require folate and B12 for both proliferation and differentiation. Folate and/or B12 deficiency have direct impact on purine and thymine metabolism and can disrupt DNA synthesis and promotes erythroblast apoptosis; this will ultimately result in ineffective erythropoiesis and anemia. Erythroblasts require substantial amounts of iron for hemoglobin biosynthesis[2]. Iron deficiency suppresses erythropoietin production via activation of the iron regulatory protein 1 (IRP1) - hypoxia inducible factor 2α (HIF2α) axis; this limits erythropoiesis under conditions of systemic iron restriction[3]. Iron deficiency also results in diminished consumption of vitamin B12 and folate, although serum levels may remain within normal limits.

In the light of this information, we hypothesized that serum levels of vitamin B12 and folate may undergo a rapid decrease in association with sudden accelerations in erythropoiesis observed in response to iron treatment; our preliminary clinical observations suggested that administration of iron supplements resulted in a substantial decrease in serum B12 levels among patients in our cohort. In this study, our intent was to determine whether serum levels of B12 and folic acid in patients diagnosed with IDA undergo change in response to iron supplements.

Material and Methods

This study is a retrospective, cross-sectional, single-center study. Our subjects included 202 female patients of reproductive age who presented to our hospital between July 2018 and July 2019 and who were diagnosed with iron deficiency anemia (IDA) according to the World Health Organization (WHO) 2001 criteria (1) and who were treated with iron supplements only. This study was approved by the ethics committee. Laboratory values for hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), white blood cells count (WBC), platelet count (Plt), as well as serum ferritin, folate and B12 levels were evaluated at presentation and again at 4–8 weeks after the treatment. Patients were diagnosed with IDA based on Hb levels <12 g/dL and serum ferritin levels <30 µg/L at presentation; we diagnosed vitamin B12 deficiency in patients with serum B12 levels <126.5 ng/L (reference range: 126.5–505 ng/L) and folate deficiency among those with folate levels <3.1 µg/L (reference range: 3.1−19.9 µg/L). Exclusion criteria included: patients diagnosed with a disease or disorder other than iron deficiency, patients diagnosed with iron deficiency secondary to malignant disease or gastrointestinal surgery, women who were pregnant or lactating at the time of presentation, women who were in menopause or who were amenorrheic, patients who were undergoing erythrocyte transfusion, patients undergoing concomitant treatment with vitamin B12, vitamin complexes and/or folic acid supplements in addition to iron therapy, and those who were not available for follow-up within the 4–8 week designated in this study.

Statistics

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL) software. Normal distribution of the data was assessed with the Kolmogorov-Smirnov test. Numerical variables that exhibit normal distribution were presented as mean ± standard deviation; those that exhibited non-normal distributions were presented as median (min-max). Categorical variables were presented as numbers and percentages. In all populations including the treatment groups (oral and intravenous administration groups), laboratory results before and after iron supplementation were assessed with generalized linear mixed model (GLMM) for the analysis of longitudinal data with repeated measurements. The relationship between the changes in the laboratory parameters after iron replacement was examined by Spearman Correlation Analysis. For the purposes of statistical analysis, *p* values <0.05 were considered to be significant.

Results

We enrolled 202 patients in our study; 51 patients (25.2%) were treated with oral iron supplementation and 151 (74.8%) were treated with IV iron replacement. The age range of the patients was between 18-55 years and their mean age was 38.7 ± 8.7 years. There was no difference in the mean age of patients that received oral iron replacement *vs.* those treated with IV iron replacement (37.4 ± 9.3 *vs*. 39.2 ± 8.5; *p* = 0.188). The Hcts of the patients who received oral iron therapy were higher at presentation compared to Hcts of patients treated with IV iron replacement (31.9 ± 4.6 *vs*. 30.2 ± 4.3; *p* = 0.015). No other laboratory findings differed significantly between the two treatment groups (Table 1).

Table 1. Patient demographics and laboratory values at presentation

Variables	Total population n = 202	Oral Iron n = 51	IV Iron n = 151	p
Age (years)	38.7 ± 8.7	37.4 ± 9.3	39.2 ± 8,5	0.188
Hb (g/dL)	9.4 ± 1.6	9.7 ± 1.7	9.3 ± 1.6	0.094
Hct (%)	30.6 ± 4.4	31.9 ± 4,6	30.2 ± 4.3	0.015*
MCV (fL)	69.1±8.6	71.1±8.9	68.5±8.4	0.061
WBC (x10^3/µl)	6.6 (2.7-15.7)	6.4(2.7-13.9)	6.7(2.7-15.7)	0.845
Plt (x10^3/µl)	319(107-4880)	324(107-4880)	319(139-573)	0.790
Ferritin (µg/L)	3.5(0.5-23.3)	3.7(1.4-23.3)	3.4(0.5-16.6)	0.074
Folate (µg/L)	8.3(3.1-24.4)	8.8(3.1-20)	8(3.4-24.4)	0.500
B12 (ng/L)	258(96-635)	276(147-526)	253(96-635)	0.115

Values are presented as mean ± standard deviation or median (min-max) as noted in the Methods.
*p < 0.05 indicates statistical significance.

Table 2. Responses to iron replacement therapy

Variables	Pre-treatment n = 202	Post-treatment n = 202	p
Hb (g/dL)	9.4 ± 1.6	11.8 ± 1.3	<0.001*
Hct (%)	30.6 ± 4.4	36.6 ± 3.5	<0.001*
MCV (fL)	69.1 ± 8.6	76.9 ± 8.4	<0.001*
WBC (x10^3 /µl)	6.6 (2.7-15.7)	6.9 (3.3-14.7)	0.025*
Plt (x10^3 /µl)	319 (107-4880)	268 (28-520)	<0.001*
Ferritin (µg/L)	3.5 (0.5-23.3)	41.1(2.4-288.8)	<0.001*
Folate (µg/L)	8.3 (3.1-24.4)	8.0 (2.1-24)	0.005*
B12 (ng/L)	258 (96-635)	244 (51-652)	<0.001*

Values are presented as mean ± standard deviation or median (min-max).
*p < 0.05 indicates statistical significance.

Table 3. Laboratory values evaluated pre- and post-treatment in patients treated with oral or IV iron replacement therapy

Variables	Oral Iron Replacement n=51			IV Iron Replacement n=151			Δp
	Pre-treatment	Post-treatment	p	Pre-treatment	Post-treatment	p	oral vs IV
Hb (g/dL)	9.7±1.7	11.6±1.6	<0.001*	9.3±1.6	11.9±1.2	<0.001*	0.004*
Hct (%)	31.9±4.6	36.8±4.5	<0.001*	30.2±4.3	36.5±3.1	<0.001*	0.023*
MCV (fL)	71.1±8.9	77.3±8.4	<0.001*	68.5±8.4	76.7±8.5	<0.001*	0.104
WBC(x10^3/µl)	6.4 (2.7-13.9)	7.2 (3.7-11.4)	0.123	6.7 (2.7-15.7)	6.7 (3.3-14.7)	0.096	0.690
Plt (x10^3/ µl)	324 (107-4880)	262 (105-461)	<0.001*	319 (139-573)	269.5 (28-520)	<0.001*	0.083
Ferritin(µg/L)	3.7 (1.4-23.3)	12.4 (2.9-66.7)	<0.001*	3.4 (0.5-16.6)	68.4 (2.4-288.8)	<0.001*	<0.001*
Folate (µg/L)	8.8 (3.1-20)	8.5 (3.1-23.9)	0.570	8 (3.4-24.4)	7.6 (2.1-24)	0.013*	0.037*
B12 (ng/L)	276 (147-526)	249 (141-428)	0.007*	253 (96-635)	243.5 (51-652)	<0.001*	0.217

Values are presented as mean ± standard deviation or median (min-max).
*$p < 0.05$ indicates statistical significance.

Overall, significant increases were detected in mean Hb, mean Hct, mean MCV, median WBC, and median serum ferritin levels in all patients treated with iron therapy (both oral and intravenous); significant decreases were observed in post-treatment median Plt, median folate, and median B12 levels in this patient cohort (Table 2).

When evaluated by treatment group, we observed significant increases in post-treatment mean Hb, Hct, and MCV and median serum ferritin levels among patients treated with oral iron replacement; significant decreases were observed in median Plt and serum B12 levels after treatment. Analogous findings were observed in response to IV iron supplementation, albeit with an additional significant decrease in median serum folate levels. Of note, more substantial increases in post-treatment mean Hb, mean Hct and median serum ferritin levels were identified among the patients treated with IV iron replacement compared to those provided with oral iron therapy; as noted above, those on IV iron therapy also responded with a significant decrease in serum folate levels. No additional statistically significant differences were noted (Table 3).

Discussion

In this study, we evaluated serum B12 and folate levels both before and after iron treatment in patients diagnosed with IDA. We found that Hb, MCV, serum ferritin, and WBC all increased, whereas serum levels of B12 and folate and Plts all decreased in response to iron treatment. Interestingly, serum folate levels responded to oral iron therapy with a small but not statistically significant decrease; by contrast, we found a larger and statistically significant decrease in serum folate levels in response to IV iron therapy. This may be because IV iron results in a more rapid mobilization of reticulocytes and promotes accelerated erythropoiesis[4] with insufficient time to correct diminishing folate levels secondary to increased utilization.

Roberts et al.[5] previously demonstrated that both serum and erythrocyte levels of folate were decreased in response to IV iron therapy together with improvements in associated megaloblastic changes in the bone marrow. They explained this observation with a focus on the iron-dependent folate biosynthetic enzyme, glutamate formimino transferase. Specifically, iron deficiency may interfere with folate metabolism by reducing the activity of this critical enzyme. Velez et al.[6] also reported decreases in serum folate levels following iron treatment in four of six iron-deficient patients. Following iron treatment, folate metabolism improved together with resolution of findings associated with folate deficiency in the bone marrow; increased uptake of folate in the bone marrow resulted in a decrease in the serum and erythrocyte folate levels. In these cases, an absolute folate deficiency could be masked by concomitant iron deficiency[4,7]. The findings from our study are consistent with these observations.

We have also found that the serum B12 levels decreased in response to both oral and IV iron treatments. Harrison[8] reported that patients diagnosed with hypochromic anemia presented with low levels of vitamin B12 associated with peripheral erythrocytes; erythrocyte B12 levels became abnormally high in response to iron treatment and only gradually returned to normal levels. Among possible explanations provided for this observation, iron deficiency may lead to diminished enzyme content within the erythrocytes which may result in diminished intake of B12 through the cell membrane and/or limitations on its use within cells[8]. This hypothesis also explains why serum B12 levels decrease following iron treatment. Morphological changes in the peripheral

blood erythrocytes and their precursors in bone marrow due to iron deficiency may mask the evidence of co-existing folate and vitamin B12 deficiencies[4]. In contrast to the findings from our study, Remacha et al.[9] reported that serum B12 and folate levels increased in response to iron treatment in a series that included 35 otherwise healthy young women. However, the authors noted that, while iron deficiency has an impact on multiple metabolic pathways including those involving B12 and folate, levels may have normalized in response to iron treatment. Similarly, in a study including 50 patients, Roberts et al.[4] found that IV iron therapy had no impact on serum B12 levels, and Metz et al.[10] reported no significant changes in serum B12 levels following intravenous iron infusion used to treat 81 patients with postnatal anemia. These discrepancies may relate to the number of patients included in each of these studies and to their individual nutritional habits.

Taken together, our results suggest that insufficient erythropoiesis due to iron deficiency limits the use of endogenous B12 and folate; the intracellular and/or surface enzymes modulated by serum iron levels may also have an impact on B12 and folate metabolism. As such, serum B12 and folate levels may appear to be normal despite ongoing and potentially inefficient use[4,8]. Furthermore, iron, folate and B12 deficiencies may co-exist[11,12]. It is critical to recognize that microcytosis associated with iron deficiency may mask the morphological findings of B12 and/or folate deficiency; a low or normal MCV determined as part of an evaluation of IDA does not fully exclude concomitant problems associated directly with B12 and/or folate. For this reason, it is critical to include an evaluation of serum B12 and folate levels in any evaluation performed to assess responses to iron treatment so as to be certain to include the possibility of these associated deficiencies. Similarly, patients who do not mount an adequate response to iron treatment, including reticulocytosis and an increase in Hb and Hct should be re-evaluated immediately to rule out B12 and/or folate deficiency.

Study Limitations

Among the limitations of this study, we were unable to compare our findings to those reported previously because, to the best of our knowledge, there are no current and comprehensive published studies that addressed the impact of iron therapy on serum B12 and folate levels. Similarly, due to the retrospective nature of the study design, peripheral blood smear and bone marrow data were not available to us at this time.

Conclusion

We conclude that deficiencies in serum levels B12, folate and iron, which are critical factors promoting erythropoiesis may effectively mask one another in a basic clinical assessment of anemia. As such, we recommend a full assessment of B12, folate and iron as part of the comprehensive diagnosis, treatment and follow-up of any patient presenting with anemia.

Conflict of Interest

The authors declare that they have no conflict of interest

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None

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