

Cobalamin Deficiency Can Mask Depleted Body Iron Reserves

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Abstract Vitamin B12 deficiency impairs DNA synthesis and causes erythroblast apoptosis, resulting in anaemia from ineffective erythropoiesis. Iron and cobalamin deficiency are found together in patients for various reasons. We have observed that cobalamin deficiency masks iron deficiency in some patients. We hypothesised that iron is not used by erythroblasts because of ineffective erythropoiesis due to cobalamin deficiency. Therefore, we aimed to demonstrate that depleted iron body reserves are masked by cobalamin deficiency. Seventy-five patients who were diagnosed with cobalamin deficiency were enrolled in this study. Complete blood counts and serum levels of iron, unsaturated iron binding capacity (UIBC), ferritin, vitamin B₁₂, and thyroid stimulant hormone were determined at diagnosis and after cobalamin therapy. Patients who had a combined deficiency at diagnosis and after cobalamin therapy were recorded. Before cobalamin therapy, we found increased serum iron levels ($126.4 \pm 63.4 \mu\text{g/dL}$), decreased serum UIBC levels ($143.7 \pm 70.8 \mu\text{g/dL}$), increased serum ferritin levels ($192.5 \pm 116.4 \text{ ng/mL}$), and increased transferrin saturation values ($47.2 \pm 23.5 \%$). After cobalamin therapy, serum iron levels ($59.1 \pm 30 \mu\text{g/dL}$), serum ferritin levels ($44.9 \pm 38.9 \text{ ng/mL}$) and transferrin saturation values ($17.5 \pm 9.6 \%$) decreased, and serum UIBC levels ($295.9 \pm 80.6 \mu\text{g/dL}$) increased. Significant differences were observed in all values ($p < 0.0001$). Seven patients (9.3 %) had iron deficiency

before cobalamin therapy, 37 (49.3 %) had iron deficiency after cobalamin therapy, and a significant difference was detected between the proportions of patients who had iron deficiency ($p < 0.0001$). This study is important because insufficient data are available on this condition. Our results indicate that iron deficiency is common in patients with cobalamin deficiency, and that cobalamin deficiency can mask iron deficiency. Therefore, we suggest that all patients diagnosed with cobalamin deficiency should be screened for iron deficiency, particularly after cobalamin therapy.

Keywords Cobalamin deficiency · Megaloblastic anaemia · Iron deficiency · Utilisation of iron · Ineffective erythropoiesis

Introduction

Deficiencies in a number of vitamins and minerals required for normal erythropoiesis (haematinics) is associated with anaemia [1]. Vitamin B₁₂ and folate (the most common haematinics) are the most prevalent forms of vitamin deficiency worldwide [1], and vitamin B₁₂ deficiency is a major public health problem [2]. Deficiency of folate or cobalamin causes megaloblastic anaemia, a disease in which pancytopenia results from differentiating haematopoietic cells that die before reaching maturity [3]. This effect is most prominent in the erythroid lineage and is termed ineffective erythropoiesis [3].

A lack of vitamin B₁₂ may be caused by insufficient intake or malabsorption of the vitamin [2]. Insufficient intake of vitamin B₁₂ is seen in vegetarians and vegans [2]. Malabsorption of vitamin B₁₂ occurs in patients suffering from a number of gastrointestinal conditions [2]. Common

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conditions are related to decreased or abolished output of gastric intrinsic factor and/or hypo- and achlorhydria, as seen in patients with destroyed gastric mucosa caused either by an autoimmune mechanism or by gastric atrophy [2]. Importantly, a recent study suggested that vitamin B₁₂ deficiency may be preceded by iron deficiency in these cases [2], and impaired iron absorption is a likely consequence [4]. However, screening and preventive measures for iron deficiency are commonly overlooked [4], and clinicians must be aware of coexisting conditions, particularly iron deficiency [5].

Based on our clinical observations and the few data about the association between cobalamin and iron deficiency, we hypothesised that cobalamin deficiency masks iron deficiency because of decreased iron utilisation in erythroid cells depending on slowed erythropoiesis. Therefore, we aimed to demonstrate that depleted iron body reserves could be masked depending on ineffective erythropoiesis in patients with cobalamin deficiency.

Methods

Patients

This study was a retrospective, cross-sectional, single centre study. Seventy-five patients diagnosed with cobalamin deficiency between January 2005 and November 2013 were enrolled. The selection of patients was laboratory based, and independent from aetiology. Patients who had folate deficiency and hypothyroidism were excluded.

Measurements

Complete blood counts and serum iron, unsaturated iron binding capacity (UIBC), ferritin, vitamin B₁₂, and thyroid stimulant hormone (TSH) levels were determined at diagnosis and after cobalamin therapy. Iron indices were assessed when vitamin B₁₂ deficiency findings disappeared (generally 1–3 months after initiating cyanocobalamin therapy).

Serum vitamin B₁₂ and TSH concentrations were measured using an electrochemiluminescence-immunoassay technique intended for use with the Elecsys reagent kit supplied by Roche Diagnostics GmbH (Mannheim, Germany), and run on Cobas e 601 immunoassay analyser (Roche Diagnostics). Serum iron and UIBC were measured by the ferrozine method (Roche Diagnostics), and serum ferritin was measured by an immunoturbidimetric method (Roche Diagnostics) and run on a Cobas e 601 immunoassay analyser. Transferrin saturation was calculated as the ratio of serum iron to total iron binding capacity \times 100. Complete blood counts of all patients were analysed with a

Coulter LH 750 haematological analyser (Beckman-Coulter, Brea, CA, USA).

Definitions

Cobalamin deficiency was defined as serum cobalamin level <100 pg/mL with macrocytosis [6], and iron deficiency was defined as a serum ferritin level <15 ng/mL and/or transferrin saturation <16 % according to the World Health Organisation criteria.

Treatments

Cyanocobalamin was used to treat vitamin B₁₂ deficiency in all patients. Patients received 1,000 μ g vitamin B₁₂ intramuscularly daily for 7 days, then 1,000 μ g weekly for 4 weeks, and 1,000 μ g every month for maintenance therapy.

Statistical Analysis

Patient characteristics were examined using descriptive statistics. Continuous variables are presented as mean \pm standard deviation (SD), and categorical variables are defined as percentages. The Chi square and *t*-tests were used to compare proportions and means for categorical and continuous variables, respectively. Variables with significant *p* values ($p < 0.05$) and marginally insignificant *p* values ($p < 0.1$) in a univariate analysis were included in a multivariate analysis. All significance tests were two-tailed. SPSS 17.0 for Windows statistical software (SPSS Inc., Chicago, IL, USA) was used for all statistical calculations.

Results

The study included 75 patients [37 (49.3 %) females and 38 (50.7 %) males; mean age, 64.3 ± 14.4 years; 65.5 ± 15.6 years for females and 63.1 ± 13.3 years for males]. The characteristics of the patients at diagnosis, and after cobalamin treatment, and comparisons between the groups are shown in Table 1.

Seven patients (9.3 %) in 75 patients had iron deficiency before cobalamin therapy, 37 patients (49.3 %) in 75 patients had iron deficiency after cobalamin therapy, and a significant difference was detected between the proportion of patients who had iron deficiency ($p < 0.0001$).

The characteristics of the 38 patients who had pure cobalamin deficiency at diagnosis and after cobalamin treatment and a comparison between the groups are shown in Table 2.

The characteristics of the 37 patients who had both iron and cobalamin deficiency at diagnosis, and after the

Table 1 Data of all the 75 patients at baseline and after cobalamin treatment

	Baseline (mean ± SD)	After treatment (mean ± SD)	<i>p</i> values
WBC ($\times 10^3/\mu\text{L}$)	5.2 ± 1.9	7.9 ± 2.8	<0.0001
RBC ($\times 10^{12}/\text{L}$)	2.29 ± 0.69	4.67 ± 0.65	<0.0001
Hb (g/dL)	9.3 ± 2.5	13.6 ± 1.8	<0.0001
Hct (%)	27.1 ± 7.6	41.1 ± 5.0	<0.0001
MCV (fL)	119.3 ± 8.3	88.7 ± 8.7	<0.0001
RDW (%)	20.2 ± 4.6	16.4 ± 3.2	<0.0001
PLT ($\times 10^9/\text{L}$)	167 ± 68	238 ± 60	<0.0001
Vitamin B ₁₂ (pg/mL)	56.9 ± 17.8	–	
TSH ($\mu\text{IU}/\text{mL}$)	2.46 ± 2.32	–	
Serum iron ($\mu\text{g}/\text{dL}$)	126.4 ± 63.4	59.1 ± 30.0	<0.0001
Serum UIBC ($\mu\text{g}/\text{dL}$)	143.7 ± 70.8	295.9 ± 80.6	<0.0001
Serum ferritin (ng/mL)	192.5 ± 116.4	44.9 ± 38.9	<0.0001
Transferrin saturation (%)	47.2 ± 23.5	17.5 ± 9.6	<0.0001

cobalamin treatment, and a comparison between the groups are shown in Table 3.

Between patients who had pure cobalamin deficiency and combine deficiency, there were significant differences in serum UIBC ($p = 0.048$), ferritin ($p = 0.013$), red blood cells (RBCs) ($p = 0.023$), haematocrit ($p = 0.049$), and mean corpuscular volume (MCV) values ($p = 0.044$) before cobalamin therapy, and in serum iron ($p < 0.0001$), UIBC ($p < 0.0001$), ferritin ($p = 0.001$), TSH ($p < 0.0001$) and MCV values ($p = 0.029$) after cobalamin therapy. There was no significant difference in RDW before and after cobalamin therapy ($p = 0.575$ and $p = 0.131$, respectively).

Discussion

Folate, vitamin B₁₂, and iron have crucial roles in erythropoiesis [7]. Erythroblasts require folate and vitamin B12 for proliferation during differentiation [7]. A deficiency of folate or vitamin B12 inhibits purine and thymidylate synthesis, impairs DNA synthesis, and causes erythroblast apoptosis, resulting in anaemia from ineffective erythropoiesis. Erythroblasts require large amounts of iron for haemoglobin synthesis [7].

Iron and cobalamin deficiency can be found together in patients depending on various reasons [2, 4, 5, 8, 9]. In our study 37 (49.3 %) of the 75 patients had an iron deficiency after cobalamin therapy. Interestingly, we detected only seven patients (9.3 %) with iron deficiency before cobalamin therapy. Cobalamin deficiency complicates the diagnosis of iron deficiency. Similarly, Atrah and Davidson

Table 2 Baseline and after cobalamin treatment data of the 38 patients who had pure cobalamin deficiency

	Baseline (mean ± SD)	After treatment (mean ± SD)	<i>p</i> values
WBC ($\times 10^3/\mu\text{L}$)	4.8 ± 1.4	7.9 ± 3.3	<0.0001
RBC ($\times 10^{12}/\text{L}$)	2.15 ± 0.68	4.61 ± 0.64	<0.0001
Hb (g/dL)	8.9 ± 2.7	14.1 ± 1.7	<0.0001
Hct (%)	26.0 ± 7.8	42.0 ± 5.1	<0.0001
MCV (fL)	121.2 ± 7.2	91.6 ± 6.3	<0.0001
RDW (%)	20.2 ± 4.9	15.6 ± 2.7	0.001
PLT ($\times 10^9/\text{L}$)	163 ± 69	225 ± 65	0.001
Vitamin B ₁₂ (pg/mL)	58.9 ± 18.0	–	
TSH ($\mu\text{IU}/\text{mL}$)	2.04 ± 1.35	–	
Serum iron ($\mu\text{g}/\text{dL}$)	123.1 ± 56.4	77.8 ± 27.5	0.008
Serum UIBC ($\mu\text{g}/\text{dL}$)	124.8 ± 63.0	244.1 ± 61.6	<0.0001
Serum ferritin (ng/mL)	208.3 ± 101.3	64.3 ± 35.3	<0.0001
Transferrin saturation (%)	50.4 ± 24.1	24.3 ± 8.1	<0.0001

Table 3 Baseline and after cobalamin treatment data of the 37 patients who had both iron and cobalamin deficiency

	Baseline (mean ± SD)	After treatment (mean ± SD)	Sig. (<i>p</i> values)
WBC ($\times 10^3/\mu\text{L}$)	5.5 ± 2.0	7.9 ± 2.3	<0.0001
RBC ($\times 10^{12}/\text{L}$)	2.65 ± 0.68	4.72 ± 0.68	<0.0001
Hb (g/dL)	10.5 ± 2.5	13.2 ± 1.8	<0.0001
Hct (%)	30.7 ± 7.4	40.1 ± 4.8	<0.0001
MCV (fL)	116.7 ± 6.9	85.8 ± 9.9	<0.0001
RDW (%)	19.4 ± 4.0	17.1 ± 3.5	0.047
PLT ($\times 10^9/\text{L}$)	193 ± 72	251 ± 54	<0.0001
Vitamin B ₁₂ (pg/mL)	59.3 ± 15.0	–	
TSH ($\mu\text{IU}/\text{mL}$)	2.58 ± 1.95	–	
Serum iron ($\mu\text{g}/\text{dL}$)	112.0 ± 66.9	40.5 ± 19.0	<0.0001
Serum UIBC ($\mu\text{g}/\text{dL}$)	164.7 ± 64.0	347.7 ± 62.3	<0.0001
Serum ferritin (ng/mL)	124.3 ± 107.3	25.6 ± 32.9	<0.0001
Transferrin saturation (%)	39.8 ± 22.5	10.7 ± 5.0	<0.0001

[4] reported that iron deficiency is a common complication in patients with long-standing pernicious anaemia and that its diagnosis and treatment are commonly neglected. Demiroğlu and Dündar [8] found that iron deficiency commonly accompanies patients with pernicious anaemia and that this is more pronounced in elderly patients.

We hypothesised that iron is not used by erythroblasts because of ineffective erythropoiesis due to cobalamin deficiency. Although an iron deficiency existed in the patients, it is found that serum iron indices were high. Our findings

support this notion, as 37 patients had a combined deficiency before cobalamin therapy. After cobalamin therapy, when ineffective erythropoiesis disappeared, serum iron levels decreased to 40.5 µg/dL, UIBC levels increased to 347.7 µg/dL, serum ferritin levels decreased to 25.6 ng/mL, and serum transferrin saturation levels decreased to 10.7 % in 37 patients who had both iron and cobalamin deficiency (Table 3).

Similarly, Hillman et al. [11]. also demonstrated that patients with pernicious anaemia prior to vitamin B₁₂ therapy show very poor Fe⁵⁹ utilisation, and that Fe⁵⁹ utilisation increases after vitamin B₁₂ therapy. Gafter-Gvili et al. [12]. reported that abnormalities in iron metabolism associated with megaloblastic anaemia rapidly reverse following vitamin B₁₂ therapy in patients with pernicious anaemia. Bessman [10] reported seven patients who had vitamin deficiencies, four with B₁₂, two with folate, and one with both. The serum transferrin saturation of these patients at admission was elevated; however, after initial vitamin therapy without iron, transferrin saturation was <15 % in four, and bone marrow in all cases was megaloblastic with increased iron stores on admission [10].

Additionally, Remacha et al. [13]. reported that serum erythropoietin levels were inappropriately low for the degree of anaemia in patients with vitamin B₁₂ deficiency compared with those with a pure iron deficiency. According to the results of our study, serum UIBC, ferritin, and MCV levels may be useful for diagnosing an iron deficiency in patients with a combined deficiency before cobalamin therapy, but we did not find a significant index that would help differentially diagnose a pure cobalamin deficiency and a combined deficiency before cobalamin therapy. As expected, we found that all iron indices were useful for diagnosing iron deficiency after cobalamin therapy. It is probably that iron deficiency emerges as obvious when normal erythropoiesis begin to instead of ineffective erythropoiesis and increase iron utilisation with cobalamin therapy.

Based on these findings, we think that decreased iron utilization of erythroid cells depending on various factors (e.g. ineffective erythropoiesis and inappropriate secretion of erythropoietin) in cobalamin deficiency mask iron deficiency. It is likely that increased iron utilization in bone marrow after cobalamin administration exposes iron deficiency, when normal erythropoiesis begins to instead of ineffective erythropoiesis.

Study Limitations

Despite its contribution to the literature, this study has some limitations. The retrospective nature of the study design prevented determination of blood picture findings of patients. It is expected that combine deficiency have dimorphic blood picture before treatment. Unfortunately, it could not be evaluated

because of our study was retrospective and data could not be reached. Secondly, bone marrow findings and status of erythropoiesis was not determined.

Conclusion

The study is important because insufficient data are available on this condition. Our results indicate that iron deficiency is common in patients with cobalamin deficiency, and we suspect that many clinicians may overlook developed iron deficiency after cobalamin therapy. Therefore, we suggest that all patients diagnosed with cobalamin deficiency should be screened for iron deficiency, particularly after cobalamin therapy.

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Conflict of interest The authors declare no conflicts of interest.

References

- McNamee T, Hyland T, Harrington J, Cadogan S et al (2013) Haematinic deficiency and macrocytosis in middle-aged and older adults. *PLoS ONE* 8:e77743. doi:[10.1371/journal.pone.0081102](https://doi.org/10.1371/journal.pone.0081102)
- Hvas AM, Nexø E (2006) Diagnosis and treatment of vitamin B12 deficiency—an update. *Haematologica* 91:1506–1512
- Koury MJ, Price JO, Hicks GG (2000) Apoptosis in megaloblastic anemia occurs during DNA synthesis by a p53-independent, nucleoside-reversible mechanism. *Blood* 96:3249–3255
- Atrah HI, Davidson RJL (1988) Iron deficiency in pernicious anaemia: a neglected diagnosis. *Postgrad Med J* 64:110–111
- Remacha AF, Sardà MP, Canals C, Queraltó JM et al (2013) Combined cobalamin and iron deficiency anemia: a diagnostic approach using a model based on age and homocysteine assessment. *Ann Hematol* 92:527–531. doi:[10.1007/s00277-012-1634-8](https://doi.org/10.1007/s00277-012-1634-8)
- Pflipsen MC, Oh RC, Saguil A, Seebusen D et al (2009) The prevalence of vitamin B₁₂ deficiency in patients with type 2 diabetes: a cross-sectional study. *JABFM* 22:528–534
- Koury MJ, Ponka P (2004) New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. *Annu Rev Nutr* 24:105–131
- Demiroğlu H, Dündar S (1997) Pernicious anaemia patients should be screened for iron deficiency during follow up. *N Z Med J* 110:147–148
- Khanduri U, Sharma A (2007) Megaloblastic anaemia: prevalence and causative factors. *Natl Med J India* 20:172–175
- Bessman D (1977) Erythropoiesis during recovery from macrocytic anemia: macrocytes, normocytes, and microcytes. *Blood* 50:995–1000
- Hillman RS, Adamson J, Burka E (1968) Characteristics of vitamin B₁₂ correction of the abnormal erythropoiesis of pernicious anemia. *Blood* 31:419–432
- Gafter-Gvili A, Prokocimer M, Breuer W, Cabantchik IZ, Hershko C (2004) Non-transferrin-bound serum iron (NTBI) in megaloblastic anemia: effect of vitamin B(12) treatment. *Hematol J* 5:32–34
- Remacha AF, Bellido M, García-Die F, Marco N et al (1997) Serum erythropoietin and erythroid activity in vitamin B12 deficiency. *Haematologica* 82:67–68