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Role of Combined Nutritional Deficiency in Microcytic Anemia: A Retrospective Study

Mikrositik Anemide Kombine Beslenme Eksikliğinin Rolü: Retrospektif Bir Çalışma

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Abstract

Objective: The mean erythrocyte volume (MCV) plays an important role in the differential diagnosis of nutritional anemia. Generally, the causes of microcytic anemia in low MCV and macrocytic anemia causes in high MCV are considered. In this study, it was aimed to examine the relationship between nutritional anemias and erythrocyte indices.

Methods: The files of patients with nutritional anemia in the pediatric hematology outpatient clinic were analyzed retrospectively. Patients whose hemogram parameters, iron, total iron binding capacity, ferritin, B12 and folic acid levels were studied were included in the study. Combined nutritional anemia was defined as the coexistence of both types of anemia (iron deficiency and B12 deficiency).

Results: A total of 407 patients, 252 (61.9%) female and 155 (38.1%) male, were included in the study. The mean age of the patients was 8.82±6.15 years. Iron deficiency anemia was found in 192 (47.2%) patients, combined nutritional anemia in 185 (45.4%) patients, and B12 deficiency anemia in 23 (5.7%) patients. MCV, iron and transferrin saturation were found to be lower in the iron deficiency anemia group compared to the group with combined nutritional anemia ($p<0.05$). However, both the iron deficiency group and the combined nutritional anemia group had microcytic anemia (respectively, MCV: 70.39 fL, MCV: 78.18 fL).

Conclusion: Combined nutritional anemia is as common as iron deficiency anemia and may present as microcytic anemia. MCV is not a guide in these patients. Therefore, B12 levels should be checked in addition to iron parameters in patients who present with microcytic anemia and are thought to have nutritional anemia.

Keywords: Mean erythrocyte volume, iron deficiency, B12 deficiency, combine nutritional anemia

Öz

Amaç: Ortalama eritrosit hacmi (MCV), beslenme anemisinin ayırıcı tanısında önemli bir rol oynar. Genellikle düşük MCV'de mikrositik anemi nedenleri, yüksek MCV'de ise makrositik anemi nedenleri ele alınır. Bu çalışmada beslenme anemileri ile eritrosit indeksleri arasındaki ilişkinin incelenmesi amaçlandı.

Yöntem: Çocuk hematoloji polikliniğinde beslenme anemisi tanısı alan hastaların dosyaları retrospektif olarak incelendi. Hemogram parametreleri, demir, total demir bağlama kapasitesi, ferritin, B12 ve folik asit düzeyleri çalışılan hastalar çalışmaya dahil edildi. Kombine beslenme anemisi, her iki anemi tipinin (demir eksikliği ve B12 eksikliği) bir arada bulunması olarak tanımlandı.

Bulgular: Çalışmaya 252'si (%61,9) kadın, 155'i (%38,1) erkek olmak üzere toplam 407 hasta dahil edildi. Hastaların yaş ortalaması 8,82±6,15 yıldı. Hastaların 192'sinde (%47,2) demir eksikliği anemisi, 185'inde (%45,4) kombine beslenme anemisi, 23'ünde (%5,7) B12 eksikliği anemisi saptandı. Demir eksikliği anemisi

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Öz

grubunda MCV, demir ve transferrin satürasyonu kombine beslenme anemisi olan gruba göre daha düşük bulundu (p<0,05). Ancak hem demir eksikliği grubunda hem de kombine beslenme anemisi grubunda mikrositik anemi mevcuttu (sırasıyla MCV: 70,39 fL, MCV: 78,18 fL).

Sonuç: Kombine beslenme anemisi, demir eksikliği anemisi kadar yaygındır ve mikrositik anemi ile ortaya çıkabilir. MCV bu hastalarda yol gösterici değildir. Bu nedenle mikrositer anemi ile başvuran ve beslenme anemisi olduğu düşünülen hastalarda demir parametrelerinin yanı sıra B12 düzeylerine de bakılmalıdır.

Anahtar Kelimeler: Ortalama eritrosit hacmi, demir eksikliği, B12 eksikliği, kombine beslenme anemisi

Introduction

Pediatric patients with anemia are frequently encountered in pediatric outpatient clinics. Because anemia is a symptom, not a disease, the underlying cause must be investigated and treated. The most common causes of anemia in children are nutritional anemia, thalassemia, and chronic disease. Nutritional anemia is the most common type of anemia worldwide. It develops due to a deficiency of micronutrients such as iron, vitamin B12, folic acid, and zinc, which play a vital role in the synthesis of hemoglobin[1,2]. In various studies conducted in Turkey, the frequency of iron deficiency anemia was reported to be in a wide range between 3.29% and 29.9%[3,4]. Vitamin B12 and folic deficiency anemia are less common than iron deficiency anemia. Specifically, in children younger than two years of age, B12 deficiency is known to cause anemia and neurological development retardation. Delay in treatment can lead to serious complications such as severe anemia and irreversible neurological damage[5]. Hence, early diagnosis and treatment of B12 deficiency anemia are important. In classifying anemia, the mean erythrocyte volume (MCV) is utilized, according to which iron deficiency causes microcytic anemia, B12, and folic acid deficiencies give rise to macrocytic anemia. In iron deficiency anemia associated with vitamin B12 deficiency, normal or low MCV levels may be observed, which may lead to a misdiagnosis of B12 deficiency[6]. This study explores the potential relationship between nutritional anemia, notably combined nutritional anemia, and MCV levels in children.

Materials and Methods

Our study was designed as a single-center retrospective study. The patients' data followed in the Pediatric Hematology Polyclinic of Manisa Celal Bayar University Faculty of Medicine between January 2015 and December 2020 were extracted from the electronic data system. It comprises patients diagnosed with anemia, whose ages vary between one month and 18 years, and whose hemogram, iron parameters, B12, and folic acid values were measured simultaneously at the time of diagnosis. Those without nutritional anemia (thalassemia trait, anemia of chronic disease, aplastic anemia, etc.) were excluded from the study. Because the study was retrospective, all patients with suspected thalassemia trait did not undergo hemoglobin electrophoresis. All patients with an MCV/red blood cell (RBC) ratio 13 were excluded from the study because of a suspicion of thalassemia. Accordingly, the lower limits for hemoglobin levels for the age groups were as follows: 11 g/dL from 6 months to 5 years, 11.5 g/dL from 5 to 12 years, and 12 g/dL from 12 to 15 years, regardless of gender. For those older than 15 years, 12 g/dL in girls and 13 g/dL in men[7]. The formulas used to obtain the MCV's lower and upper limits were as follows: For the former, 70+ age for those younger than 10 years and 80 fL for the older ones, and 84+ (0.6x age) for the latter. In patients younger than one year of age, the lower limit for MCV was accepted as 73 fL and the upper limit as 85 fL[2,4,8]. The lower limits for ferritin were taken as 12 mcg/L, and those for folic acid and vitamin B12 were assumed to be 3 ng/mL and 200 pg/mL, where B12 values below 120 pg/mL referred to B12 deficiency[4,9]. The patients were divided into four groups by age: Infants (0-2 years), toddlers (2-6 years), schoolchildren (6-12 years), and adolescents (12-18 years). The term "combined nutritional anemia" was defined as the coexistence of both types of anemia (iron deficiency and B12 deficiency).

Statistical Analysis

Statistical analysis was performed using the Statistical Program for Social Sciences (SPSS) 23 package program. All statistical tests (Mann-Whitney U and Students' t-tests for the comparison of non-normally and normally distributed continuous measurements, respectively) were performed at the $p<0.05$ significance level. Categorical data were evaluated using the chi-square test. Ethical approval for the current study was obtained from the Scientific Research Ethics Committee of Manisa Celal Bayar University the approval number: 20.478.486/692, dated: 30/12/2020.

Results

Of 407 patients, 252 (61.9%) were female and 155 (38.1%) were male, with a mean age of 8.82±6.15 years. In particular, 21.9% (n=89) were infants, 20.4% (n=83) toddlers, 14.5% (n=59) schoolchildren, and 43.2% (n=176) adolescents. Isolated iron deficiency anemia was the most common type of nutritional anemia in 192 (47.2%) patients, followed by combined nutritional anemia in 185 patients (45.4%). Only 23 patients had isolated B12 deficiency anemia (Table 1). Most patients in our study consisted of patients who were followed up in other outpatient clinics for complaints unrelated to anemia or who were diagnosed with anemia incidentally. Overall, 181 (44.4%) patients had no anemia-related problems, but the remaining 226 patients (55.6%) had such complaints. The most frequent issues were fatigue (50.8%), pallor (29.2%), and anorexia (27.8%) (Table 2). Analysis of the distribution of subgroups of nutritional anemia by age groups revealed that combined nutritional anemia was most common in infants and adolescents (57.3% and 48.6%, respectively), and iron deficiency anemia was most frequent in toddlers and schoolchildren (61.5% and 61%, respectively) (Table 3). The comparison of hemogram parameters of patients with iron deficiency anemia and those with combined nutritional anemia revealed that while the B12 value was lower in the group with combined nutritional anemia, MCV, iron, and transferrin saturation were higher ($p<0.05$). No significant difference was found in terms of other parameters. The mean MCV value was 70.39 fL in the iron-deficient group and 72.18 fL in the combined nutritional anemia group. Both groups had microcytic anemia (Table 4). The comparison of the B12 deficiency anemia and combined nutritional anemia groups revealed that the latter was associated with lower MCV, MCH, MCHC, iron, ferritin, and TS but higher RBC, red cell distribution width, TDBK, B12, and platelet count (PLT)/MCH levels ($p<0.05$) (Table 5). There were 213 patients with B12 levels <200 pg/mL. When the MCV levels of patients with B12 <120 pg/mL (n=82) and those with 120-200 pg/mL (n=131) were compared, 75.1±9.8 fL and 71.8±8.2 fL were found, respectively ($p=0.1$). Macrocytosis was detected in only one (4.3%) of 23 patients with isolated B12 deficiency. Eight (34.7%) patients had microcytosis and 14 (60.9%) patients had normocytosis. Five (21.7%) patients in the isolated B12 deficiency anemia group were under one year old.

Table 1. Nutritional anemias detected in patients

Type of nutritional anemia	n=407	%
Iron deficiency anemia	192	47.2
Iron deficiency + B12 deficiency anemia*	185	45.4
B12 deficiency anemia	23	5.7
Iron deficiency and folic acid deficiency anemia	4	1
Iron deficiency-B12 deficiency-folic acid deficiency anemia	2	0.5
B12 deficiency and folic acid deficiency anemia	1	0.2

*: Iron deficiency + B12 deficiency anemia; combined nutritional anemia

Table 2. The complaints of the patients

Complaints	n=226	%
Fatigue	115	50.8
Pallor	66	29.2
Anorexia	63	27.8
Anorexia and fatigue	34	15
Fatigue and pallor	20	8.8
Dizziness	13	5.7
Palpitation	7	3
Motor retardation	5	2.2
Hair loss	5	2.2
Failure to thrive	4	1.7
Pica	4	1.7
Fatigue and hair loss	3	1.3
Bloody stool	3	1.3
Breath holding spell	3	1.3
Nose bleeding	1	0.4

Discussion

Nutritional anemia develops because of the deficiency of micronutrients such as iron, vitamin B12, folic acid, and zinc, which play crucial roles in hemoglobin synthesis. While it is the primary type of nutritional anemia, iron deficiency anemia is also the most prevalent type of anemia in childhood[1,4]. The prevalence of iron deficiency anemia among children in low- and middle-income countries varies between approximately 35% and 90%[10,11]. Nutritional vitamin B12 deficiency anemia, similar to iron deficiency anemia, is a significant health problem, particularly in developing countries. Some studies have reported that the incidence of vitamin B12 deficiency anemia changes between 22% and 65% in regions with low socioeconomic status[12,13]. In our study, iron deficiency anemia was the predominant form (47.2%), followed by combined nutritional anemia (185 patients-45.4%), and isolated B12 deficiency anemia (23 patients-5.7%). Among 185 patients with combined nutritional anemia, 61 had B12 levels below 120 pg/mL. Even though nutritional anemia patients often present with different complaints, such as weakness, loss

Table 3. Distribution of nutritional anemias by age groups					
Age groups	**n**	**%**	**Type of nutritional anemia**	**n**	**%**
0-2 years	89	21.9	Iron deficiency B12 deficiency Iron deficiency + B12 deficiency	32 6 51	36 6.7 57.3
2-6 years	83	20.4	Iron deficiency B12 deficiency Iron deficiency + B12 deficiency	51 5 27	61.5 6 32.5
6-12 years	59	14.5	Iron deficiency B12 deficiency Iron deficiency + B12 deficiency Iron deficiency + B12 deficiency + folic acid deficiency B12 deficiency + folic acid deficiency	36 4 17 1 1	61 6.8 28.8 1.7 1.7
12-18 years	176	43.2	Iron deficiency B12 deficiency Iron deficiency + B12 deficiency Iron deficiency+ folic acid deficiency Iron deficiency + B12 deficiency + folic acid deficiency	73 8 90 2 3	41.7 4.5 48.6 1.1 1.7
0-18 years	407	100		407	

Table 4. Laboratory parameters of iron deficiency anemia and combined nutritional anemia			
	Iron deficiency anemia (n=192)	**Combined nutritional anemia (n=185)**	**p-value ***
WBC (/mm³)	7897.70±2334.46	7792.37±2475.12	0.671
RBC	4.37±0.43	4.28±0.57	0.098
HB (g/dL)	9.42±1.46	9.43±1.68	0.983
HTC (%)	30.60±3.76	30.50±4.63	0.818
MCV (fL)	70.39±7.44	72.18±8.69	**0.032**
MCH (pg)	21.78±3.31	22.46±3.80	0,066
MCHC (g/dL)	30.67±1.92	30.95±1.99	0.166
RDW (%)	17.53±3.27	17.6±3.15	0.159
PLT (/mm³)	357208.33±124947.06	351054.05±111148.63	0.614
PLT/MCV (mm³/fL)	5175.74±2091.34	4958.55±1834.47	0.285
PLT/MCH (mm³/pg)	16981.18±7755.34	16228.52±6505.28	0.309
FE (ug/dL)	21.37±10.16	24.88±10.16	**0.006**
TDBK (ug/dL)	431.21±69.31	426.34±77.48	0.520
TS (%)	5.03±2.53	5.95±3.78	**0.005**
Ferritin (ng/mL)	6.25±3.55	6.33±3.59	0.830
B12 (pg/mL)	325.58±113.38	133.50±37.36	**0.000**
Folic acid (ng/mL)	10.86±5.05	11.23±6.08	0.525

*: $p<0.05$, FE: Iron, TDBK: Total iron-binding capacity, TS: Transferrin saturation, WBC: White blood cell, RBC: Red blood cell, MCV: Mean erythrocyte volume, HTC: Hydrochlorothiazide, MCH: Mean corpuscular hemoglobin concentration, PLT: Platelet count

Table 5. Laboratory parameters of B12 deficiency anemia and combined nutritional anemia

	B12 deficiency anemia (n=23)	Combined nutritional anemia (n=185)	p-value *
WBC (/mm³)	7940.86±2081.47	7792.37±2475.12	0.458
RBC	3.95±0.72	4.44±0.62	**0.004**
HB (g/dL)	10.00±1.54	9.43±1.68	0.082
HTC %	31.38±5.00	30.50±4.63	0.190
MCV (fL)	80.22±7.12	72.18±8.69	**<0.001**
MCH (pg)	25.85±2.87	22.46±3.80	**<0.001**
MCHC (g/dL)	32.22±1.23	30.95±1.99	**0.001**
RDW (%)	15.04±2.37	17.06±3.15	**<0.001**
PLT (/mm³)	347521.73±153135.96	351054.05±111148.63	0.513
PLT/MCV (mm³/fL)	4386.02±2002.31	4958.55±1834.47	0.078
PLT/MCH (mm³/pg)	13654.21±6264.97	16228.52±6505.28	**0.036**
FE (ug/dL)	82.11±69.19	24.88±10.16	**<0.001**
TDBK (ug/dL)	353.46±72.04	426.34±77.48	**<0.001**
TS (%)	22.61±14.91	5.95±5.78	**<0.001**
Ferritin (ng/mL)	16.52±13.63	6.33±3.59	**<0.001**
B12 (pg/mL)	107.04±41.78	133.50±37.36	**0.004**
Folic acid (ng/mL)	10.90±5.67	11.23±6.08	0.984

*: $p<0.05$, FE: Iron, TDBK: Total iron-binding capacity, TS: Transferrin saturation, WBC: White blood cell, RBC: Red blood cell, MCV: Mean erythrocyte volume, HTC: Hydrochlorothiazide, MCH: Mean corpuscular hemoglobin concentration, PLT: Platelet count

of appetite, pallor, and pica, those with mild anemia may have no complaints, which in turn renders the diagnosis incidental. In fact, in our study, most patients (44.4%) had no complaints, and these patients applied to other clinics for various unrelated reasons. In a prospective study that focused on nutritional anemia, the most common symptoms were cited as anorexia (71%), fatigue (45%), and pica (36.3%), respectively[14]. In our study, the most common complaints were fatigue (50.8%), pallor (29.2%), and anorexia (27.8%).

Nutritional anemia is more frequent in infancy and adolescence, during periods of rapid growth and increased nutritional needs. We found that anemia was most prevalent among the 12-18-year-old group (43.2%). Our finding is in harmony with the results of an earlier study, which also revealed that this age group had higher deficiencies of iron, folate, and vitamin B12 than the others[15]. In a similar study that explored 1120 randomly selected children aged between 12 and 16 years, anemia was identified in 5.6% of them, of whom the majority had iron deficiency anemia (59%), and the remaining had (41%) combined nutritional anemia[16]. In contrast, in our study, the preponderance had combined nutritional anemia (48.6%), followed by iron deficiency anemia (41.7%). More importantly, combined nutritional anemia was detected at a high rate in the 0-2 age group (57.3%). Because neurological development is rapid in this age group, it is vital to detect and treat B12 and iron deficiency.

Mean erythrocyte volume, one of the erythrocyte indices, is one of the most important parameters used in the classification of anemia. In anemic patients, high MCV indicates macrocytosis and low MCV indicates microcytosis. Often, isolated B12 deficiency anemia manifests itself as macrocytic anemia, but some studies have presented evidence against it. A retrospective study on children and adolescents with anemia indicated that only 3.5% of patients with isolated B12 deficiency had macrocytosis[15]. We reached a similar result: Macrocytosis was present in only one of 23 isolated cases of B12 deficiency anemia (4.3%). Remarkably, macrocytosis was rare in isolated B12-deficient anemia. A study investigating the relationship between the level of B12 deficiency and MCV could not find a significant difference between the two groups of patients: Those having B12 levels of 200-300 pg/mL and the others below 200 pg/mL (The respective MCVs were 80.12±7.25 and 80.57±5.33)[17]. In our study, we did not find a significant difference between the MCV levels of patients with B12 levels <120 pg/mL and those with 120-200 pg/mL (75.1 fL and 71.8 fL, respectively). Overall, these findings demonstrate that MCV levels may be misleading in estimating isolated B12 deficiency anemia. In addition, macrocytosis may be masked when B12 deficiency anemia is accompanied by thalassemia, iron deficiency anemia, or anemia of chronic disease, resulting in underdiagnosis of B12 deficiency anemia[6,18]. In fact, a study that investigated MCV levels in combined nutritional anemia found the mean MCV levels were low (59.8±6.6 fL)[14]. Likewise, in our study, microcytosis was observed not only in iron deficiency anemia but also in combined nutritional anemia (MCV 70.39±7.44 and 72.18±8.69, respectively). Overall, a patient presenting with microcytic anemia need not have solely isolated iron deficiency anemia, as iron deficiency anemia can coexist with B12 deficiency anemia. Because MCV alone is not a complete guide in combined nutritional anemia, some studies have examined specific parameters such as MCV/PLT or MCH/PLT. A study performed on adults discovered that combined nutritional anemia patients had higher platelet levels and lower MCV levels than those with pure iron deficiency anemia. Consequently, the authors found that the PLT/MCV ratio was significantly higher when iron deficiency was copresent with vitamin B12 deficiency, recommending the measurement of vitamin B12 levels in patients with iron deficiency anemia with a high PLT/MCV ratio[19]. However, our results indicated no difference between the two groups regarding the above-mentioned parameters. On the other hand, when we compared patients with combined nutritional anemia with those with B12 deficiency anemia, the former was associated with an elevated PLT/MCH ratio. This increase may be due to the higher incidence of thrombocytosis and lower MCH levels in patients with iron deficiency.

B12 deficiency can cause not only megaloblastic anemia and severe neurological deficits. A study on 15 B12 deficiency anemia patients (with a mean age of 11.7 months), of whom 46.6% presented with epileptic seizures, observed neurodevelopmental retardation, pallor, hypotonia, and anorexia[20]. In the same vein, another study reported motor and developmental retardation in infants whose mothers were vegan, vegetarian, or had a B12-restricted diet[21]. In congruence with the research above, our outpatient clinic examination identified motor retardation in five patients with B12 deficiency anemia. Two of the patients presenting with motor retardation had isolated B12 deficiency anemia, whereas the other three were from the combined nutritional anemia group.

Study Limitations

The fact that the research was conducted in a single center can be considered as one of the limitations of the study. It is also a retrospective study, but we used a standard study form to collect data, and the same clinician performed the data collection.

Conclusion

Especially in microcytic anemia due to nutritional reasons, iron deficiency is likely to be accompanied by B12 deficiency. Early diagnosis and appropriate treatment of vitamin B12 deficiency are necessary because they can lead to severe neurological and hematological problems if left untreated. Therefore, it is essential to measure serum vitamin B12 levels and iron parameters in patients presenting with microcytic anemia.

Ethics

Ethics Committee Approval: Ethical approval for the current study was obtained from the Scientific Research Ethics Committee of Manisa Celal Bayar University the approval number: 20.478.486/692, dated: 30/12/2020.

Informed Consent: Retrospective study.

Authorship Contributions

Concept: A.T.Y., Design: A.T.Y., Data Collection or Processing: Ş.A.A., Analysis or Interpretation: Ş.A.A., A.T.Y., Literature Search: Ş.A.A., A.T.Y., Writing: Ş.A.A., A.T.Y.

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