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RESEARCH ARTICLE

Evaluation of Complete Blood Count Parameters in Patients with Methylmalonic Acidemia

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ABSTRACT

Objective: We aimed to evaluate the frequency of pathological changes in blood parameters and their relationship with serum creatinine and glomerular filtration rate (GFR) in patients with methylmalonic acidemia.
Methods: Demographic and laboratory data of 46 patients diagnosed by acylcarnitine, urine organic acid, and/or molecular analyses for methylmalonic acidemia were evaluated. In all patients, complete blood counts, serum iron concentrations, serum iron-binding capacity, vitamin B12 and folate concentrations, and serum creatinine tests were performed during the period when the patients were metabolically stable.
Results: Among the 46 patients with anaemia, 54.3% had anaemia of chronic disease, 19.6% had iron deficiency anaemia. Bicytopenia was detected in 17.4%. There was a negative correlation between serum creatinine levels and leucocyte, lymphocyte, erythrocyte, and platelet counts. GFR values were positively correlated with haemoglobin value, leukocyte, lymphocyte, erythrocyte, and platelet counts.
Conclusions: The presence of anaemia, neutropenia, thrombocytopenia, and erythrocyte volume changes in patients with methylmalonic acidemia apart from the metabolic attack period is a situation that reveals the necessity of a detailed nutritional evaluation of patients. Evaluation of renal function in the presence of haematological complications and taking precautions if signs of renal failure are noted may prevent worsening of complications.

Keywords: Methylmalonic acidemia, anemia, thrombocytopenia, renal failure, bone marrow

INTRODUCTION

Methylmalonic aciduria is a disorder of methylmalonic acid (MMA) and cobalamin (cbl) metabolism that can be caused by different genetic problems. Isolated methylmalonic aciduria is usually a result of partial [mut (-)] or complete [mut (0)] deficiency of methylmalonyl-CoA mutase, a mitochondrial enzyme caused by mutations in the MUT gene (1). However, cblA, cblB, and cblD deficiencies leading to metabolic problems in the synthesis or transport of adenosyl-cobalamin, the cofactor of methylmalonyl-CoA mutase, can cause isolated methylmalonic acidemia. In cblC, cblD, and cblF deficiencies, which are among the disorders of cobalamin metabolism, methylmalonic aciduria and homocystinuria are observed together.

Patients usually develop lethargy, tachypnoea, vomiting, dehydration, acute metabolic acidosis, ketosis, and hyperammonemia shortly after birth. In the absence of appropriate treatment, coma and death due to hyperammonemic encephalopathy may occur. If patients survive the first metabolic attack, recurrent episodes of metabolic decompensation triggered by catabolic processes, including infection, vaccination, and teething, are observed during follow-up (2). Haematological abnormalities including anaemia, leukopoenia, neutropenia, thrombocytopenia, and pancytopenia, are detected especially during metabolic attacks caused by bone marrow suppression. In most patients, multisystem complications secondary to methylmalonic aciduria, including developmental delay, optic atrophy, and renal impairment, develop (2). It has been suggested that secondary metabolic changes triggered by the accumulation of toxic metabolites, including propionyl-CoA, 2-methylcitric acid, and MMA, are among the causes of these long-term abnormalities (2).

Individuals with isolated methylmalonic aciduria are at risk of renal failure (3). The course of chronic renal failure is worsened by secondary complications of renal failure such as anaemia, arterial hypertension, renal osteodystrophy, and hyperparathyroidism (4). Patients with chronic kidney disease may not be able to use the iron stores in their body effectively and therefore may require additional iron therapy. With further deterioration of renal function, erythropoietin

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production in the kidneys may decrease, and patients may require erythropoietin treatment (5).

Anaemia, leucopoenia and thrombocytopenia have been reported during acute metabolic decompensation in patients with methylmalonic acidemia, propionic acidemia, or isovaleric acidemia (6). In this study, we aimed to evaluate the variety and frequency of pathological changes in blood parameters and the relationship between these changes and serum creatinine and glomerular filtration rate (GFR) in patients with methylmalonic acidemia during periods when they did not experience metabolic attacks.

MATERIALS AND METHODS

The demographic and laboratory characteristics of 46 patients who were diagnosed as having an acylcarnitine profile by tandem mass spectrometry (MSMS), organic acid profile by gas chromatography-mass spectrometry (GC-MS), and/or methylmalonic acidemia by molecular analysis between 1995 and 2022 in the Department of Paediatric Nutrition and Metabolism, Istanbul Faculty of Medicine were evaluated. The Ethics Committee of Istanbul Faculty of Medicine approved the study (file number 2023/1500, date: 25/08/23). Written informed consent was obtained from all participants or their legal guardians after the study procedure was explained.

In all patients, complete blood count, serum iron concentration, serum iron binding capacity, vitamin B12 and folate concentrations, and serum creatinine tests performed using conventional methods during the period when the patients were metabolically stable were evaluated from the file notes. A haemoglobin value below the 5th percentile of the normal value determined for that age (7), a serum ferritin value < 12 mcg/L below the age of 5 years and < 15 mcg/L above the age of 5 years (8), and a transferrin saturation below 15% have been accepted as iron deficiency anaemia (9). Chronic disease anaemia has been defined with decreased plasma iron concentration, decreased total iron binding capacity, decreased transferrin saturation, and normal or increased ferritin concentration (10). While cytopenia in two blood cell populations is defined as bicytopenia, the combination of anaemia, neutropenia, and thrombocytopenia is defined as pancytopenia. The glomerular filtration rate (GFR) was calculated using the Schwartz formula. The stages of renal failure were determined according to the glomerular filtration rates of the patients (11).

Mean, standard deviation, median, minimum, maximum, and ratio values were used in the descriptive statistics of the data. The distribution of variables was measured using the Kolmogorov–Smirnov test. The Spearman correlation analysis test was used to analyse quantitative independent data. SPSS 28.0 software was used in the analyses.

RESULTS

The mean age was 14.7±8.7 (median: 13.1, range: 1-41.1) years in 46 patients, including 31 males and 15 females from 37 families. The mean follow-up period was 14.5±7.5 (range: 11 months-28.7 years; median: 13.1 years). Extended newborn screening was the diagnostic method for three patients within the first month of life. Due to sibling history, 3 patients were diagnosed in the first week of life, 1 patient in the prenatal period, and one at 13 years of age. Thirty-eight patients were diagnosed due to symptomatic presentation. Fifteen symptomatic patients were diagnosed within the first month of life. The age at symptomatic presentation ranged from 1 month to 6 years (median 2 years) (Table 1).

Of the 46 patients with anaemia, 25 (54.3%) had anaemia of chronic disease and 9 (19.6%) had iron deficiency anaemia. Bicytopenia was found in 8 (17.4%) of 46 children. Among these, anaemia and thrombocytopenia were found in 2 children, anaemia and neutropenia in 4 children, anaemia and lymphopenia in 1 child, and thrombocytopenia and lymphopenia in 1 child. No patient had pancytopenia.

When renal failure was evaluated according to glomerular filtration rate, stage 1 renal failure was found in 27 patients, stage 2 renal failure in 8 patients, stage 3 renal failure in 7 patients, and stage 4 renal failure in 4 patients. Age was positively correlated with serum creatinine levels and negatively correlated with GFR, leucocyte, lymphocyte, and platelet counts. There was a negative correlation between serum creatinine levels and leucocyte, lymphocyte, erythrocyte, and platelet counts. GFR values were positively correlated with haemoglobin value, leukocyte, lymphocyte, erythrocyte, and platelet counts (Table 2).

DISCUSSION

In patients with methylmalonic acidemia and other organic acidemias, cytopenia is a condition that usually occurs during periods of acute decompensation. However, patients may also experience anaemia, neutropenia, or thrombocytopenia outside these periods. The pathophysiology underlying bone marrow involvement in methylmalonic acidemia is thought to involve multiple factors, such as the direct toxic effect of accumulated organic acids and other metabolites, micronutrient deficiencies, and complex mitochondrial dysfunction, which are related to impaired energy production and oxidative stress (6, 12, 13).

Although anaemia, neutropenia, thrombocytopenia, and pancytopenia have been frequently evaluated in propionic acidemia, few studies have evaluated the prevalence of these complications in patients with MMA. Kölker et al. reported that the mean haemoglobin level in patients with MMA was 40% below the reference range. On average, the leukocyte count of patients was 7% below the reference range. The platelet count was 6% below the reference range in patients with MMA (14). Another study reported that 37 (28.0%) of 132 patients diagnosed with cobalamin C deficiency had anaemia (15). Leukopoenia was found in 21/35, anaemia in 11/33, and thrombocytopenia in 15/30 of 45 patients with isolated methylmalonic acidemia (16). In Tavil et al. an anaemia was found in all 11 patients with a diagnosis of MMA, and one of them was reported to have pancytopenia. Anaemia

Table 1: Demographic data and complete blood count parameters of patients with methylmalonic acidemia.

Patient	Gender	Diagnosis method	Age at diagnosis	Follow-up (years)	Renal Insufficiency stage	Hb (g/dl)	PLT (10^3/µl)	PNL (10^3/µl)
1	M	Symptomatic	1 m	7.1	1	13.6	351	3.5
2	M	Symptomatic	1 m	6.2	1	13.2	469	3.2
3	M	Symptomatic	1 m	13.1	1	10.4	142	3.6
4	F	Sibling history	1 m	26.5	1	10.2	205	4.7
5	M	Symptomatic	1 m	9.2	1	12.8	337	3.9
6	F	Symptomatic	1 m	3.4	1	12.3	431	2.7
7	M	Symptomatic	1 m	10.1	1	12.6	379	4.2
8	M	Symptomatic	1 m	11.7	1	10.9	330	4
9	M	Symptomatic	3 m	8.2	1	14	296	4.7
10	M	NBS	1 m	9.6	1	14.2	381	3.7
11	F	Symptomatic	10 m	16.7	1	14.6	186	3.1
12	F	Symptomatic	1 m	7.8	1	14.1	325	6.1
13	F	NBS	1 m	13.5	1	11.6	259	4.6
14	M	Symptomatic	6 y	26	1	16.4	255	5.2
15	M	Symptomatic	1 m	0.8	1	11.5	284	3.5
16	M	Symptomatic	1 m	1.5	1	8.9	494	1.9
17	F	Symptomatic	2 m	9.6	1	10.1	355	7.8
18	M	Symptomatic	7 m	7.8	1	12	284	10.4
19	F	Symptomatic	2 m	7.2	1	9.9	389	5.1
20	M	Symptomatic		6.5	1	13.9	241	3.1
21	M	Symptomatic	8 m	12.7	1	11.4	280	3.4
22	M	Symptomatic	8 m	15	1	14.6	293	4.1
23	F	Symptomatic	1 m	13.1	1	13.8	317	5.8
24	M	Sibling history	1 m	11.7	1	37	207	5.3
25	M	NBS	1 m	10.3	1	12.3	331	5.1
26	F	Symptomatic	1 m	0.9	1	9.25	179	1.64
27	F	Symptomatic	15 m	16.3	1	12	216	3.1
28	M	Sibling history	1 m	4.9	2	8.6	275	5
29	M	Symptomatic	4 m	23.3	2	11.1	159	2
30	M	Symptomatic	1 m	22.5	2	12.2	162	2.5
31	M	Symptomatic	8 m	5.6	2	9.3	268	2
32	M	Symptomatic	1.5 y	11	2	8.2	225	6
33	M	Symptomatic	22 m	17.1	2	17.5	250	5.9
34	F	Symptomatic	1 m	28.7	2	10	262	4.5
35	F	Symptomatic	6 m	19.3	2	11	141	2.8
36	F	Symptomatic	1 m	14.6	3	11.7	190	1.3
37	M	Symptomatic	1 m	17.0	3	10.5	167	2
38	M	Symptomatic	2 m	23	3	13	332	3.1
39	M	Sibling history	13 y	28.1	3	15.3	245	5.6
40	M	Symptomatic	5 m	22.1	3	10	197	2.6

Table 1: Continued.

Patient	Gender	Diagnosis method	Age at diagnosis	Follow-up (years)	Renal Insufficiency stage	Hb (g/dl)	PLT (10^3/µl)	PNL (10^3/µl)
41	M	Sibling history	1 m	10.7	3	11.9	204	3.8
42	M	Symptomatic	2 m	23	3	11	337	2.6
43	M	Symptomatic	4 m	23.4	4	9.9	199	1.8
44	F	Symptomatic	5 m	19.9	4	6.5	125	1.7
45	M	Symptomatic	15 m	22	4	11	202	4.2
46	F	Symptomatic	3 m	26	4	10.2	362	5

F: Female, M: Male, NBS: Newborn screening, Hb: Haemoglobin, PLT: Platelet, PNL: Polymorph nuclear leucocytes; m: months, y: years; *prenatal diagnosis

Table 2: Correlations of age, serum creatinine level, glomerular filtration rate, and complete blood count parameters in patients with methylmalonic acidemia

		Age	Cr	GFR	Hb	PLT	PNL	LYM	Leu	RBC
Age	**CC**	1,000	,737**	-,555**	0,025	-,449**	-0,007	-,705**	-,455**	-0,036
	p		0,000	0,000	0,869	0,002	0,961	0,000	0,001	0,815
Cr	**CC**	,737**	1,000	-,917**	-0,171	-,488**	-0,135	-,553**	-,479**	-,302*
	p	0,000		0,000	0,256	0,001	0,370	0,000	0,001	0,044
GFR	**CC**	-,555**	-,917**	1,000	,301*	,431**	0,213	,355*	,361*	,403**
	p	0,000	0,000		0,047	0,004	0,165	0,020	0,016	0,007
Hb	**CC**	0,025	-0,171	,301*	1,000	0,185	,317*	0,026	0,165	,820**
	p	0,869	0,256	0,047		0,217	0,032	0,867	0,272	0,000
PLT	**CC**	-,449**	-,488**	,431**	0,185	1,000	,338*	,381**	,463**	,316*
	p	0,002	0,001	0,004	0,217		0,022	0,010	0,001	0,035
PNL	**CC**	-0,007	-0,135	0,213	,317*	,338*	1,000	0,005	,596**	,306*
	p	0,961	0,370	0,165	0,032	0,022		0,976	0,000	0,041
LYM	**CC**	-,705**	-,553**	,355*	0,026	,381**	0,005	1,000	,560**	-0,022
	p	0,000	0,000	0,020	0,867	0,010	0,976		0,000	0,886
Leu	**CC**	-,455**	-,479**	,361*	0,165	,463**	,596**	,560**	1,000	0,176
	p	0,001	0,001	0,016	0,272	0,001	0,000	0,000		0,248
RBC	**CC**	-0,036	-,302*	,403**	,820**	,316*	,306*	-0,022	0,176	1,000
	p	0,815	0,044	0,007	0,000	0,035	0,041	0,886	0,248	

Cr: Serum creatinin, GFR: Glomerular filtration rate, Hb: Haemoglobin, PLT: Platelet, PNL: Polymorph nuclear leucocytes, LYM: Lymphocyte, Leu: Leucocyte, RBC: Erythrocyte, CC: Correlation coefficient, p: 2-tailed significance; *Correlation is significant at 0.05, **Correlation is significant at 0.01

was evaluated as chronic disease in eight patients and iron deficiency in two (17). As observed in other case series of patients with methylmalonic acidemia, anaemia was found at a high rate in our patient group. Anaemia was found in 34 (73.9%) patients, 25 of whom were evaluated as anaemia of chronic disease and 9 were evaluated as iron deficiency anaemia. This situation reveals that anaemia is a frequent morbidity and should be emphasised sensitively during follow-up of patients. Two patients whose blood samples were evaluated outside the acute metabolic attack period had anaemia and thrombocytopenia, four had anaemia and neutropenia, one had anaemia and lymphopenia, and one had lymphopenia and thrombocytopenia. Low blood cell counts in patients outside the metabolic attack period are associated with metabolite accumulation, nutrient deficiencies, and mitochondrial dysfunction (6, 12, 13). Therefore, blood count parameters should be considered as an important factor in patient follow-up and treatment organisation.

It is known that bone marrow function in patients with renal failure is suppressed, iron stores cannot be effectively used, and haematological complications occur with decreased erythropoietin production as renal failure progresses (5). When renal failure and blood parameters of patients followed up with a diagnosis of methylmalonic acidemia were examined, GFR was positively correlated with haemoglobin concentration, erythrocyte count, platelet count, leukocyte count, and lymphocyte count. Serum creatinine levels were negatively

correlated with erythrocyte, leukocyte, lymphocyte, and platelet counts. Anaemia was detected in eight of the 11 patients with stage 3 and 4 renal failure. Patients with methylmalonic acidemia should be carefully monitored for the development of anaemia, especially after renal function begins to be affected. Renal failure should be taken into consideration when designing treatment for anaemia, and different treatment options should be evaluated.

The fact that the study was conducted in a single centre is a limitation factor in terms of reflecting the practises of only one centre. The small number of patients is another limitation.

CONCLUSION

Patients diagnosed with organic acidemia frequently present with pancytopenia requiring blood transfusion during metabolic decompensation, which resolve spontaneously within a few weeks (18). In cases of resistant pancytopenia, it is important to exclude other bone marrow pathologies, such as hypoplasia, aplasia, hemophagocytic lymphohistiocytosis, and myelodysplastic syndrome. In addition, because blood tissue has a high regeneration rate, its nutrient requirements are higher than those of other tissues. This situation causes haematopoietic tissue to be easily affected by nutritional deficiencies (19). In patients, nutrient deficiencies may develop because special nutritional therapies are applied in which natural protein intake is restricted and protein intake is interrupted during metabolic attack periods to prevent the accumulation of toxic substances (20). We believe that the presence of anaemia, neutropenia, thrombocytopenia, and erythrocyte volume changes in patients with methylmalonic acidemia outside the metabolic attack period is a condition that highlights the necessity of detailed nutritional evaluation of patients. Evaluation of renal function in the presence of haematological complications in patients with methylmalonic acidemia and taking precautions when signs of renal failure are present may be a step to prevent worsening of complications. Further research is needed to better understand the mechanisms underlying haematological complications in this patient group and to develop effective treatments for these patients.

Ethics Committee Approval: This study was approved by the ethics committee of Istanbul Faculty of Medicine approved the study (file number 2023/1500, date: 25/08/23).

Informed Consent: Written consent was obtained from all participants or their legal guardians.

Peer Review: Externally peer-reviewed.

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