ORIGINAL ARTICLE

Diagnostic dilemma of patients with methylmalonic aciduria: Experience from a tertiary care centre in Pakistan

Hafsa Majid,[1] Lena Jafri,[2] Aysha Habib Khan,[3] Zeba Zulfiqar Ali,[4] Azeema Jamil,[5] Nasir Yusufzai,[6] Midhat Fatimah,[7] Bushra Afroze[8]

Abstract

Objective: To determine the frequency of disorders leading to methylmalonic acidurias.

Methods: This cross-sectional study was conducted from January 2013 to April 2016 at the Aga Khan University Hospital, Karachi, and comprised patients diagnosed with methylmalonic acidurias based on urine organic acid analysis. Clinical history and biochemical data was collected from the biochemical genetics laboratory requisition forms. Organic acid chromatograms of all the subjects were critically reviewed by a biochemical pathologist and a metabolic physician. For assessing the clinical outcome, medical charts of the patients were reviewed. SPSS 19 was used for data analysis.

Results: Of the 1,778 patients 50(2.81%) were detected with methylmalonic acidurias. After excluding patients with non-significant peaks of methylmalonic acidemia, 41(2.31%) were included in the final analysis. Of these, 20(48.7%) were females, while the overall median age was 11.5 months (interquartile range: 6-41.5). On stratification by type of disorders leading to methylmalonic acidurias, 9(22%) had methylmalonic acidemia, 12(29%) had Cobalamin-related remethylation disorders, nonspecific methylmalonic acidurias in 16(39%), while 2(5%) each had succinyl coenzyme A synthetase and Vitamin B12 deficiency. respectively.

Conclusion: Screening tests, including urine organic acid, provided valuable clues to the aetiology of methylmalonic acidurias.

Keywords: Methylmalonic aciduria, Methylmalonyl-CoA mutase deficiency, Cobalamin related remethylation disorders, Vitamin B 12 deficiency, Pakistan. (JPMA 68: 510; 2018)

Introduction

Methylmalonic acidurias (MMAurias) are a heterogeneous group of inherited metabolic disorders (IMDs) leading to increased urinary methylmalonic acid excretion.[1] The aetiology of MMAurias includes isolated and combined with hyperhomocystenaemia.[2] Isolated MMAuria is caused by complete or partial deficiency of the enzyme methylmalonyl-CoA mutase (MCM) usually with normal serum methionine concentration.[3,4] In intracellular Cobalamin-related remethylation disorders (Cb1-RD), MMAuria and hyperhomocystenaemia are present. A tricarboxylic acid (TCA) cycle disorder; Succinate-CoA ligase (SUCL) also present as MMAuria but with TCA cycle markers (fumarate, succinate, malate, and 2-ketoglutarate) identified on urine chromatography.[5] Vitamin B12 deficiency is a non-IMD cause of MMAuria combined with hyperhomocytenaemia.[6,7]

The age of presentation of MMAurias varies from neonatal period to adulthood. Patients present with vomiting, dehydration, hypotonia, lethargy, encephalopathy, seizures, intellectual disability (ID), psychomotor regression, movement disorders and failure to thrive.[8,9]

MMA is identified as the most common organic acidemia in Asians in the sub-continent in literature.[10,11] Reports published from Pakistan have shown high incidence of MMA but the aetiology of MMAurias has not been described.[12,13] The current study was planned to determine the frequency of disorders leading to MMAuria diagnosed by urine organic acid (UOA) analysis and the outcome.

Patients and Methods

The cross-sectional study was conducted from January 2013 to April 2016 at Biochemical Genetic Laboratory (BGL) in the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine and Department of Paediatrics and Child Health, Aga Khan University (AKU), Karachi. It comprised patients diagnosed with MMAurias based on UOA analysis. Peaks were categorised into marked, moderate and small based on comparison with internal standard. Cases with very small peaks of methylmalonic acidemia (MMA) compared to internal standard, on UOA chromatograms were excluded. Clinical history and biochemical data was collected from the BGL

[1-3,5-7]Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, [4,8]Department of Paediatrics & Child Health, Aga Khan University Hospital, Karachi.

Correspondence: Bushra Afroze. Email: bushra.afroze@aku.edu

requisition forms. Biochemical data related to UOA, plasma amino acid (PAA), serum folic acid (FA), serum B12, plasma total homocysteine (tHcy), plasma lactate (LA), plasma ammonia (NH3), blood pH, and mean corpuscular volume (MCV) were reviewed. Ethical exemption was obtained from the institutional ethical review committee.

For UOA analysis, spot-urine samples were collected without preservative, while for PAA analysis, blood samples were collected in lithium heparin tubes. Samples were separated and stored at -20°C until analysis. Quantitative analysis of PAA was performed by cation exchange-high performance liquid chromatography on Biochrom 30+ Amino acid analyser (Biochrom, US). Qualitative evaluations of organic acids in urine was performed by gas chromatography mass spectrometry (GCMS) on Agilent GCMS system (Agilent Technologies, US) using ethyl acetate, derivatisation by bis (trimethylsilyl) acetamide compound and internal standard in UOA testing used is 3,3 dimethyl glutaric acid. Semi quantitation of methylmalonate, methyl citrate, 3 hydroxy (3 OH) isovalerate and tiglylglycine was done using formula: area under curve of metabolite/area under curve of internal standard × concentration of internal standard. It was reported in pseudounits (PU).[14]

During the period of study, BGL also participated in proficiency testing programme for UOA and PAA by European Research Network for evaluation and improvement of screening, diagnosis and treatment of Inherited Disorders of Metabolism (ERNDIM, UK) and College of American Pathologists (CAP) for all other analytes.

Organic acid chromatograms of all patients with MMAurias were critically reviewed by a biochemical pathologist and a metabolic physician. Cases with very small peaks of methylmalonic acid were excluded (Figure-1).

All patients with MMAuria were grouped as below based on the result of biochemical investigations:

MMA due to methylmalonyl CoA mutate deficiency — normal FA, tHcy, B12 and methionine; Cb1-RD defects — high tHcy, low plasma methionine, normal serum B12 and FA;

a. SUCL deficiency — presence of fumarate, succinate, 2-ketoglutarate and malate with MMAuria;

b. B12 deficient — serum B12 <200pg/ml; and nonspecific MMAuria — tHcy, FA and B12 data not available.

For assessing the clinical outcome, medical charts of patients followed by metabolic physician were reviewed while outside referrals followed by general paediatrician were contacted by phone and history of clinical presentation, details of treatment received and mortality was collected on a pre-structured questionnaire.

Frequency and percentage was generated for gender, while median with interquartile ranges (IQR) were calculated for quantitative parameters. Frequency and percentage of patients with different types of MMAurias was generated. Comparison between Cb1-RD defects, MMA and nonspecific MMAuria was performed by Kruskal Wallis test, taking $p < 0.05$ as significant. B12 and SUCL deficiencies were excluded from comparison analysis due to the small sample size. SPSS 19 was used for data analysis.

Results

Of the 1,778 patients who underwent UOA analysis, 50(2.81%) were detected with MMAurias. However, 9(18%) patients with small peaks of methylmalonate were excluded. Frequency of MMAuria, as such, was 41(2.3%). Of these, 21(51.2%) samples were received from Sindh, 14(34%) from Punjab, 5(12%) from Khyber Pakhtunkhwa (KP), and 1(2.43%) from Baluchistan. Of those detected with MMAuria, median age at the time of diagnosis was 11.5 (IQR: 6-41.5) months; 20(48.7%) being female. Lethargy and ID were the most common clinical features, followed by failure to thrive, seizures, hypotonia and vomiting. Metabolic acidosis, anemia, hyperammonemia, lactic acidosis and hyperhomocytenemia were common biochemical findings (Table).

The median MMA levels on UOA were 352 PU (IQR: 107.7-1229). Methylcitrate was detected in 32(78%) patients, 3-OH isovalerate in 38(93%), 3-OH propionate 25(60%), tiglylglycine in 16(35%) and propionylglycine in 4(7.5%). Hypomethionenaemia was detected in 18(45%) and hyperglycinaemia favouring MMA was detected in 3(7.5%) patients.

Median age of presentations in patients with Cb1-RD defects, MMA and nonspecific MMAurias were 96 months (IQR: 19-144), 11.5 months (IQR: 5.8-15.5) and 6 months (IQR: 2.7-16.2) respectively ($p<0.001$) (Figure-1). Lethargy, seizures, developmental delay and mental retardation were statistically significantly ($p <0.05$) in Cb1-RD defects, MMA and nonspecific MMAuria (Figure-2).

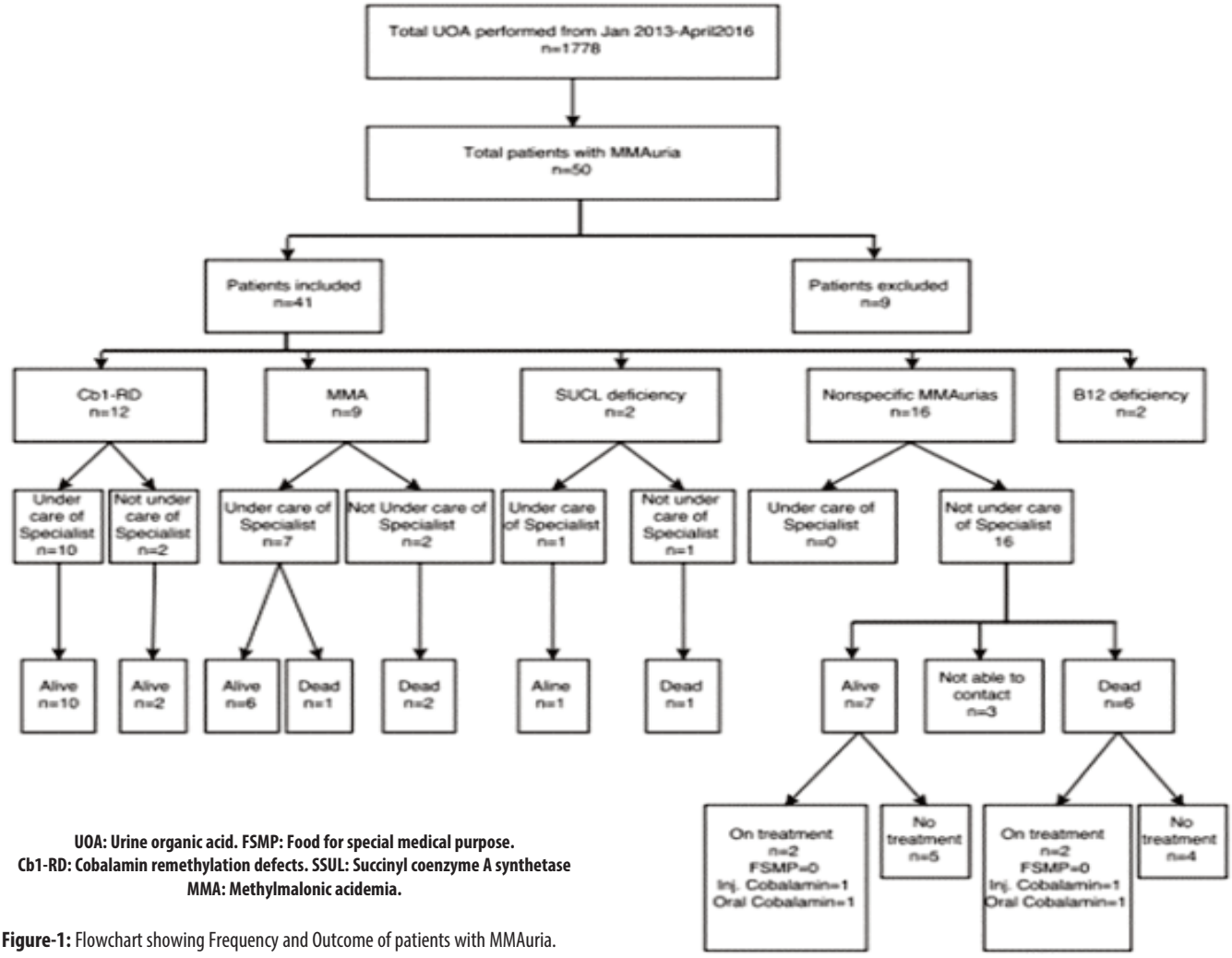
Among the biochemical parameters in the MMA, Cb1-RD and non-specific MMAuria groups, tHcy, MCV, methylmalonate, 3OH isovalerate, glycine and alanine were significantly different ($p<0.05$).

Besides, 19(46.34%) patients were followed by metabolic physician at AKUH and the remaining 22(53.66%) were

Table: Biochemical Parameters [Median (Interquartile range)].

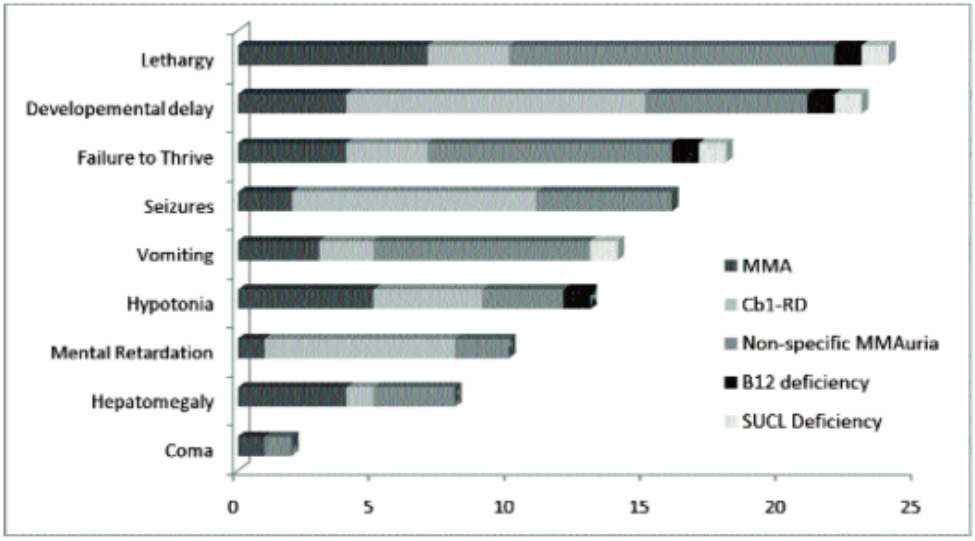
Biochemical Parameter (Reference intervals)	Total Patients n=41	Cb1-RD def n=12	MMA n=9	Non-specific MMAuria n=16	P-value
Blood pH (7.35-7.45)	7.32 (7.16-7.46)	7.48 (7.44-7.49)	7.27 (7.15-7.33)	7.33 (7.1-7.47)	0.152
Lactic Acid (0.3-11 mmol/l)	2.7 (1.48-9.2)	1.6 (1.2-3)	3.3 (1.6-22.1)	7.45 (1.78-19.5)	0.316
Plasma Ammonia (Neonate <100 µmol/l, >1 month <40 µmol/l)	56.6 (39.8-90.2)	43.6 (35.9-75.5)	68.7 (15.3-136.1)	85.5 (63.1-162.8)	0.164
MCV (Age specific Reference Range)	86.7 (80.3-102.3)	101 (83-108)	82 (80-84.7)	NA	0.073
Serum Folic Acid (2.6-12.2 ng/ml)	20 (13.8-24)	24 (19.8-24)	12.9 (10.6-19.3)	NA	0.053
Vitamin B12 (>201pg/ml)	750 (250-1057)	946 (412.7-1140)	276 (201-925)	NA	0.14
Total Homocysteine (5-12 umol/l)	58.6 (16.8-141.2)	85.6 (58.6-197.5)	9.5 (4.2-16.7)	NA	0.001
Urine Methylmalonate	352 (107.7-1229)	143 (83.4-265)	1184 (460-3655)	503 (107-1616)	0.046
Urine Methyl citrate	42.7 (18.7-125.5)	31 (5.8-160.5)	40 (15-274)	56 (29-184)	0.554
Urine 3 OH isovalerate	12.8 (6.4-81.7)	6.1 (3.1-8.9)	16 (12.5-346)	21 (8-91)	0.024
Urine Tiglylglycine	1.2 (0.42-7.5)	0.7 (0.3-3.6)	11 (0.6-18)	1.4 (0.18-5.8)	0.296
Plasma Methionine	7 (4- 11.5)	7 (4 - 10)	5 (4-11.5)	9.5 (6.2-14.5)	0.521
Plasma Glycine	259 (170-348)	268 (184-338)	179 (122.5-264)	383 (256-553)	0.032
Plasma Alanine	216 (155-340)	300 (226-380)	161 (55-206)	170 (136-279)	0.008

MCV: Mean corpuscular volume. Cb1-RD: Cobalamin remethylation defects. MMA: Methylmalonic acidemia. PU: Pseudounits.



UOA: Urine organic acid. FSMP: Food for special medical purpose.
Cb1-RD: Cobalamin remethylation defects. SSUL: Succinyl coenzyme A synthetase
MMA: Methylmalonic acidemia.

Figure-1: Flowchart showing Frequency and Outcome of patients with MMAuria.



RD: Cobalamin remethylation defects
SUCL: Succinyl coenzyme A synthetase
MMA: Methylmalonic acidaemia.

Figure-2: Clinical characteristics.

followed in different clinical settings by general paediatricians. Among those under care of metabolic physicians, 11(50%) were Cb1-RD, 6(27.27%) were MMA and 1(4.5%) with SUCL and B12 deficiency, respectively. All patients with non-specific MMAurias with incomplete biochemical evaluation were outside referrals. Mortality in the outside referrals was high compared to the group followed by metabolic physicians [9(43%) vs 1(5%)]. In non-specific MMAuria group treatment was started in 7(44%) patients only.

Discussion

MMAuria is one of the common biochemical features observed in the UOA analysis in our lab. Evaluation of the disorders leading to MMAuria is imperative for the correct diagnosis, appropriate treatment and better outcome of the patients.[15,16] Biochemical parameter including tHcy, serum B12, FA and PAA analysis is essential for the diagnostic evaluation of MMAuria.[17,18] In the present study ID was common in patients with Cb1-RD defects compared to other patients. This could be due to the younger age of presentation in MMA deficiency group, when ID is difficult to access clinically. Methylmalonate and other metabolites on UOA were higher in the MMA than in Cb1-RD defects. The tHcy levels were high in Cb1-RD patients while glycine was higher in MMA patients.

Among the 16 patients with MMAuria referred from outside, complete biochemical findings were not available as patients were not further investigated to determine the aetiological defect. The treatment prescribed to this group pointed to the lack of understanding about the difference in the treatment of MMA, Cb1-RD and SUCL deficiency. High mortality was observed in such patients. Both MMA and Cb1-RD are treatable disorders, if diagnosed early and treated appropriately.[19-21] The reason for this high mortality is likely inadequate and incomplete evaluation for the aetiology of MMAuria leading to inappropriate treatment. These findings advocate the need to improve health services and education of healthcare providers to advance the understanding of IMDs, training of human resources, expansion of and equality of access to diagnostic tests.

Lack of enzymatic analysis for MMA, complementation studies for Cb1-RD and molecular studies of the patients due to the local non-availability of these advance tests is a limitation of our study. We were also unable to categorise 16 patients into an aetiological classification of MMAuria due to the non-availability of the relevant biochemical parameters.

Conclusion

MMAuria is a biochemical finding present in patients with MMA, Cb1-RD, SUCL deficiency and serum B12 deficiency. Thus all patients with MMAuria should be further investigated with PAA, tHcy, B12 and FA levels for the correct diagnosis. A correct diagnosis allows clinicians to prescribe appropriate treatment, leading to better outcome.

Disclaimer: The abstract was presented in the Society for Inborn Errors of Metabolism Conference, 2016, at Rome, Italy.

Conflict of Interest: None.

Source of Funding: None.

References

1. Vaidyanathan K, Narayanan MP, Vasudevan DM. Organic acidurias: an updated review. Indian J Clin Biochem. 2012; 26: 319-25.

2. Fowler B, Leonard JV, Baumgartner MR. Causes of and diagnostic approach to methylmalonic acidurias. J Inherit Metab Dis. 2008; 31: 350.
3. Manoli I, Sloan JL, Venditti CP. Isolated Methylmalonic Acidemia. 2005 Aug 16 [Updated 2016 Jan 7]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2016. (Cited on March 16, 2017). Available from URL: http://www.ncbi.nlm.nih.gov/books/NBK1231/.
4. Suormala T, Baumgartner MR, Coelho D, Zavadakova P, Kozich V, Koch HG, et al. The cblD defect causes either isolated or combined deficiency of methylcobalamin and adenosylcobalamin synthesis. J Biol Chem. 2004; 279: 42742-9.
5. Ostergaard E. Disorders caused by deficiency of succinate-CoA ligase. J Inherit Metab Dis. 2008; 31: 226-9.
6. Froese DS, Gravel RA. Genetic disorders of vitamin B 12 metabolism: eight complementation groups--eight genes. Expert Rev Mol Med. 2010; 12: e37.
7. Higginbottom MC, Sweetman L, Nyhan WL. A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia and neurologic abnormalities in a vitamin B12-deficient breast-fed infant of a strict vegetarian. N Engl J Med. 1978; 299: 317-23.
8. Deodato F, Boenzi S, Santorelli FM, Dionisi-Vici C. Methylmalonic and propionic aciduria. Am J Med Genet C Semin Med Genet. 2006; 142C: 104-12.
9. Fernandes JS, J.-M.; Berghe G.v.d.; Walter JH. (Eds.). Heidelberg, Germany. Inborn Metabolic Diseases Diagnosis and Treatment. . XXII. 4th ed. 2006: pp 49-59.
10. Hori D, Hasegawa Y, Kimura M, Yang Y, Verma IC, Yamaguchi S. Clinical onset and prognosis of Asian children with organic acidemias, as detected by analysis of urinary organic acids using GC/MS, instead of mass screening. Brain Dev. 2005; 27: 39-45.
11. Song YZ, Li BX, Hao H, Xin RL, Zhang T, Zhang CH, et al. Selective screening for inborn errors of metabolism and secondary methylmalonic aciduria in pregnancy at high risk district of neural tube defects: a human metabolome study by GC-MS in China. Clin Biochem. 2008; 41: 616-20.
12. Afroze B, Lakhani L, Naz F, Somani S, Yunus ZM, Brown N. Challenges identified in the management of patients with inherited metabolic disorders-A five year experience from Pakistan. Egyptian Journal of Medical Human Genetics. 2016; 17: 259-64. (could not find any abbreviation for journal name)
13. Cheema HA, Malik HS, Parkash A, Fayyaz Z. Spectrum of Inherited Metabolic Disorders in Pakistani Children Presenting at a Tertiary Care Centre. J Coll Physicians Surg Pak. 2016; 26: 498-502.
14. Nancy W. Wentworth. Quality Assurance and Calibration Methods. In: Harris DC. Quantitative chemical analysis. 7th Edition ,New York. Macmillan; 2010. p 90-91.
15. Briani C, Dalla Torre C, Citton V, Manara R, Pompanin S, Binotto G, et al. Cobalamin deficiency: clinical picture and radiological findings. Nutrients. 2013; 5: 4521-39.
16. Zwickler T, Haege G, Riderer A, Hörster F, Hoffmann GF, Burgard P, et al. Metabolic decompensation in methylmalonic aciduria: which biochemical parameters are discriminative? J Inherit Metab Dis. 2012; 35: 797-806.
17. Baumgartner MR, Fowler B. Vitamin B12 disorders. In: Blau, N., Duran, M., Gibson, K.M., Dionisi-Vici, C, editors.Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases. Springer Berlin Heidelberg; 2014. p. 205-18.
18. Baumgartner MR, Hörster F, Dionisi-Vici C, Haliloglu G, Karall D, Chapman KA, et al. Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. Orphanet J Rare Dis. 2014; 9: 130.
19. Carrillo-Carrasco N, Chandler RJ, Venditti CP. Combined methylmalonic acidemia and homocystinuria, cblC type. I. Clinical presentations, diagnosis and management. J Inherit Metab Dis. 2012; 35: 91-102.
20. Leonard JV. The management and outcome of propionic and methylmalonic acidaemia. J Inherit Metab Dis. 1995; 18: 430-4.
21. Huemer M, Diodato D, Schwahn B, Schiff M, Bandeira A, Benoist JF, et al. Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency. J Inherit Metab Dis. 2017; 40: 21-48.