Newborn Screening for Methylmalonic Aciduria by Tandem Mass Spectrometry: 7 Years' Experience From Two Centers in Taiwan

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Background: The clinical course of methylmalonic aciduria (MMA) is fulminant in neonates and emergency management is necessary to save lives. It is therefore very important to differentiate affected from unaffected neonates immediately when there are abnormal results regarding MMA in newborn screening.

Methods: Between January 2002 and December 2008, 598,522 newborns were screened for MMA by 2 neonatal screening centers: the Chinese Foundation of Health and the Taipei Institute of Pathology. A total of 22 newborns were referred to confirmatory medical centers, and 7 were confirmed as having MMA. The initial propionylcarnitine (C3) level, C3/acetylcarnitine (C2) ratio, plasma ammonia, liver function tests, blood pH and bicarbonate were compared between the true-positive and false-positive groups.

Results: The C3/C2 ratio and plasma ammonia were markedly higher in the true-positive MMA group ($p < 0.0001$). Blood gas pH ($p = 0.029$), bicarbonate ($p = 0.019$), and aspartate aminotransferase ($p = 0.005$) also significantly differed between these 2 groups.

Conclusion: Referred newborns with elevated plasma C3/C2 ratios > 0.4 or ammonia levels > 200 μg/dL should be highly suspected of having MMA. [*J Chin Med Assoc* 2010;73(6):314–318]

Key Words: methylmalonic aciduria, newborn screening, Taiwan, tandem mass spectrometry

Introduction

Methylmalonic aciduria (MMA) comprises a heterogeneous group of disorders, which are characterized by accumulation of methylmalonate in the body due to deficiency of methylmalonyl coenzyme A mutase (MCM, EC 5.4.99.2) or defects in the uptake, transport or synthesis of 5'-deoxyadenosylcobalamin, the cofactor for MCM.[1,2] Clinical manifestations of classical MMA include metabolic acidosis, lethargy, vomiting, dehydration, hepatomegaly, hyperammonemia, neurological deterioration, and hypotonia.[3] Patients with *mut*^{0} (undetected MCM activity) have the worst prognosis. Most of these patients might have very-early-onset signs and symptoms that occur even before the results of newborn screening (NBS) are available, and die immediately or survive with significant neurodevelopmental disability.[4–6]

Because of the fulminant clinical course of MMA in neonates, emergency treatment such as energy

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supply management, antibiotics or even hemodialysis is usually necessary to save lives. Therefore, immediate differentiation of affected from unaffected neonates when there are abnormal results in MMA NBS is very important. However, the results of confirmatory tests such as urinary organic acid analysis, mutation analysis, or repeated tandem mass spectrometry (MS/MS) are usually not available immediately during such emergencies.

Therefore, we wanted to establish if there were any differences between affected and unaffected newborns by comparing the initial screening results with routine biochemical data when the newborns were referred to confirmatory medical centers. Here, we report the experience of 7 years of NBS for MMA in Taiwan, to establish if we can make an immediate accurate diagnosis of MMA by analyzing the initial laboratory data of these newborns.

Methods

Between January 2002 and December 2008, 598,522 newborns were screened for MMA using dried blood spots (DBSs) by MS/MS at the Chinese Foundation of Health and the Taipei Institute of Pathology. DBSs were obtained from newborns after 48–72 hours of life and 24–48 hours of feeding. The DBSs were analyzed by the Micromass Quattro Micro API Mass Spectrometer (Waters Corp., Milford, MA, USA) or PerkinElmer MS/MS System (PerkinElmer Inc., Waltham, MA, USA). The levels of propionylcarnitine (C3) and acetylcarnitine (C2) were measured during MMA screening. The cut-off (borderline) values were set at > 7 μM for C3 and > 0.25 for C3/C2 ratio at the Chinese Foundation of Health, and > 6 μM for C3 and > 0.2 for C3/C2 ratio at the Taipei Institute of Pathology. The positive cut-off values were established at > 10 μM for C3 and > 0.5 for C3/C2 ratio at both centers. If a screening result was greater than or equal to the positive cut-off value, the newborn was referred immediately to a confirmatory medical center. If a screening result was greater than or equal to the borderline cut-off, but less than the positive cut-off, repeat blood sampling was requested. When the second test was still outside the normal range, the newborn was referred to a confirmatory medical center.

After admission to the confirmatory referral centers, plasma ammonia, plasma amino acid analysis, urine organic acid analysis, liver function tests, and blood gas analysis were performed. MCM enzyme activity and mutation analysis were performed when MMA was found by urinary organic acid analysis.

The referred newborns were divided into a true-positive group (affected) and a false-positive group (unaffected). The affected group was defined by presence of methylmalonic acid by urine organic acid analysis, with decreased MCM activity by enzyme assay. The unaffected group was defined by normal urine organic acid analysis without abnormal clinical manifestations. Initial C3 level, C3/C2 ratio, plasma ammonia, liver function tests, blood pH and bicarbonate from blood gas analysis, gestational age, and percentile of birth body weight (BBW) were compared between the 2 groups. Categorical data were analyzed by Fisher's exact test, and continuous data by Mann–Whitney *U* test. A p value < 0.05 was of statistical significance. The statistical software package used was SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Among the 598,522 newborns screened by the 2 centers in the 7-year period, 22 patients were referred. Seven of these were confirmed to have *mut*^{0} MMA with undetected MCM activity, and the mutation analysis in 4 patients was: c.323G > A/c.1741C > T, c.1280G > A/c.1280G > A, c.1280G > A/c.754_755insA, and c.982C > T/c.982C > T. The other 3 patients were admitted to other hospitals, so the results of mutation analysis were not available. All of our affected patients were found to have hyperglycinemia by plasma amino acid analysis, with a huge peak of methylmalonic acid by urine organic acid analysis. Two of 15 unaffected newborns were admitted to other hospitals (due to personal reasons); therefore, the data from these 2 newborns were not available. All of the 7 MMA patients already had metabolic decompensation when they were admitted to the confirmatory medical centers. Their clinical manifestations included poor activity, poor appetite, vomiting, and shortness of breath. Out of 13 unaffected newborns, only 1 was found to have poor activity and feeding; later, she was diagnosed with sepsis, with *Staphylococcus sciuri* in blood culture.

The initial screening results of the above 7 patients and their routine biochemical data are briefly summarized in Table 1. Gestational age, BBW, initial C3 level, C3/C2 ratio, plasma ammonia, liver function tests, blood gas pH and bicarbonate level were analyzed in the affected and unaffected groups (Table 2). All results showed significant differences except for gestational age, BBW, initial C3 levels and alanine aminotransferase (ALT). However, when the BBW of these newborns was adjusted to percentile of BBW,

Table 1. Laboratory characteristics of the affected group with methylmalonic aciduria

	Normal values	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Range of the false-positive group
C3 (μmol/L)	< 7	19.18	12.44	16.65	12.39	7.7	10.1	14.9	8.2–22.6
C3/C2 ratio	< 0.25	1.18	0.81	0.58	1.13	0.546	0.55	0.627	0.12–0.387
Ammonia (μg/dL)	90–150	239	427	465	344	632	695	488	35–125
Blood gas pH	7.31–7.41	7.299	7.292	7.468	7.23	7.17	7.374	7.41	7.305–7.564
Bicarbonate (mmol/L)	16–24	12.3	10.3	20.3	19.7	9.7	13.9	17.1	14–24.5
AST (IU/L)	15–60	31	44	113	135	191	47	59	19–77
ALT (IU/L)	1–25	20	21	32	34	27	9	25	6–94

C3 = propionylcarnitine; C2 = acetylcarnitine; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Table 2. Statistical comparison between affected and unaffected newborns

	MMA	*n*	Mean ± SD	*p*
GA (d)	No	15	261.47 ± 26.15	0.670
	Yes	7	270.00 ± 9.75	
BBW (g)	No	15	3,256.67 ± 1,021.33	0.062
	Yes	7	2,817.86 ± 131.93	
C3 (μmol/L)	No	15	11.55 ± 3.66	0.217
	Yes	7	13.34 ± 3.91	
C3/C2 ratio	No	15	0.26 ± 0.08	< 0.0001
	Yes	7	0.77 ± 0.28	
Ammonia (μg/dL)	No	13	63.00 ± 23.72	< 0.0001
	Yes	7	470.00 ± 157.22	
Blood gas pH	No	13	7.44 ± 0.08	0.029
	Yes	7	7.32 ± 0.10	
Bicarbonate (mmol/L)	No	13	19.21 ± 3.27	0.019
	Yes	7	14.76 ± 4.34	
AST (IU/L)	No	13	35.31 ± 15.11	0.005
	Yes	7	88.57 ± 59.37	
ALT (IU/L)	No	13	20.23 ± 23.46	0.081
	Yes	7	24.00 ± 8.41	

MMA = methylmalonic aciduria; SD = standard deviation; GA = gestational age; BBW = birth body weight; C3 = propionylcarnitine; C2 = acetylcarnitine; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

the unaffected group had a significantly higher percentile than the affected group ($p = 0.001$).

Discussion

The incidence of MMA in Taiwan, based on MS/MS NBS of 598,522 infants over a 7-year period, was approximately 1 in 85,000, which is slightly lower than that in Japan (~1 in 50,000 newborns), but significantly higher than that in Germany (~1 in 250,000 newborns).[7,8] About one third of referred newborns were confirmed to have MMA in our MS/MS NBS program.

C3 is a universal primary marker for MMA screening. It alone is sensitive enough to detect classical-type MMA, but lacks 100% sensitivity for milder forms of MMA or defects of cobalamin metabolism.[9,10] Furthermore, C3 is one of the most frequent analytes found to be responsible for false-positive results in MS/MS screening.[11] To improve specificity and sensitivity, the C3/C2 ratio usually has been used as a secondary parameter.[9] Our study revealed that C3/C2 ratio was much more reliable than C3 in differentiating the affected from the unaffected newborns. There was an overlap of C3 levels, but no overlap of C3/C2 ratio between the 2 groups (Figure 1). All of the affected patients were found to have a C3/C2 ratio > 0.4,

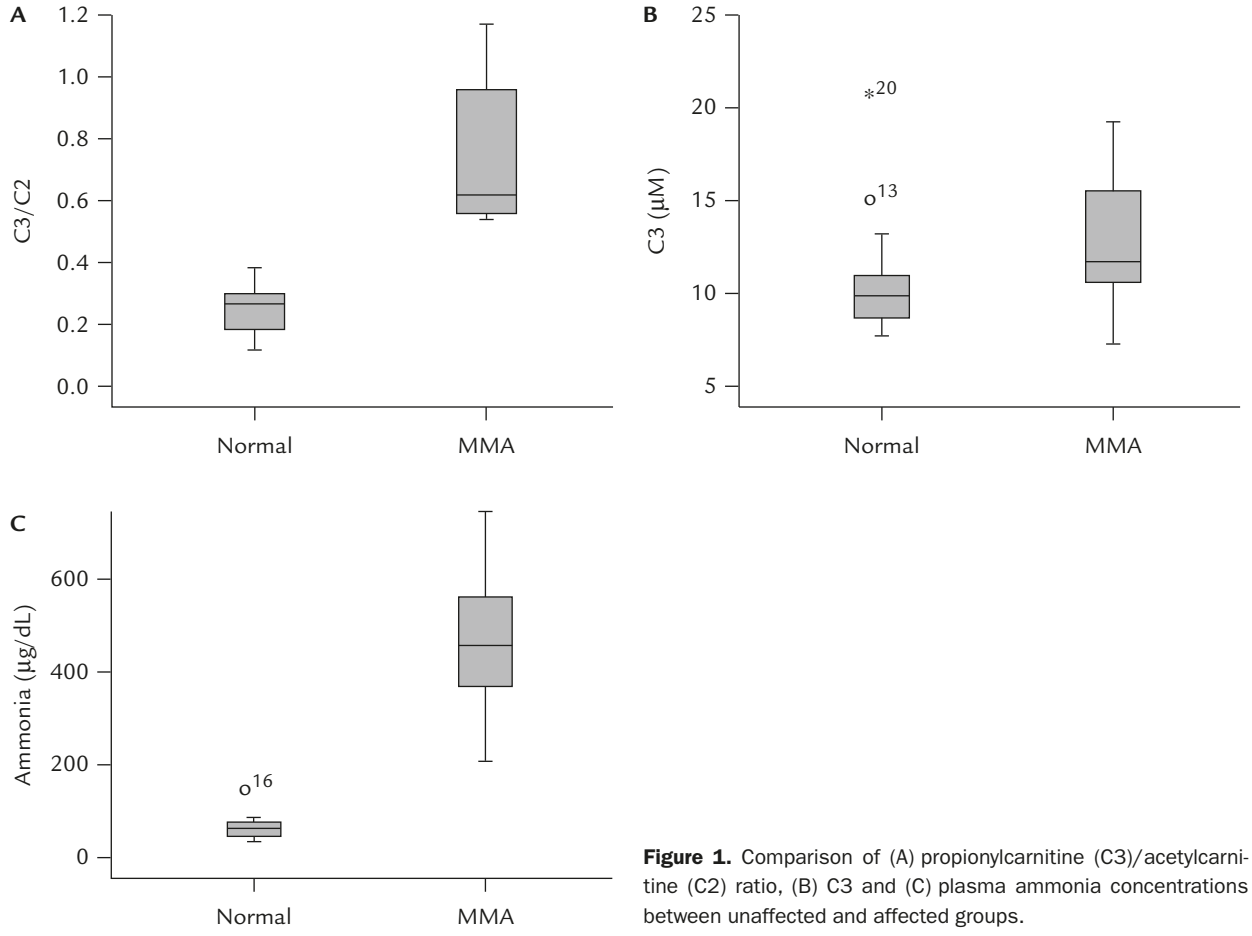

Figure 1. Comparison of (A) propionylcarnitine (C3)/acetylcarnitine (C2) ratio, (B) C3 and (C) plasma ammonia concentrations between unaffected and affected groups.

and all the unaffected patients were found to have a ratio <0.4. In our MMA screening program, the cut-off values for C3/C2 ratio in our 2 NBS centers were 0.2 and 0.25, respectively. Ten unaffected newborns would have been referred and no affected patient would have been missed if we had only used C3/C2 ratio as the primary parameter. In the MMA screening program in Germany, the cut-off value for the C3/C2 ratio was set at 0.39.[8] If we had used the German cut-off, none of the affected patients would have been missed (all patients' C3/C2 ratios were >0.5) and none of the unaffected newborns would have been picked up and referred. This could reduce the number of laboratory analyses, personnel costs for repeat tests, and considerable anxiety for parents. Therefore, we believe that we should reevaluate whether or not C3/C2 ratio could replace C3 as the primary parameter in our MMA NBS.

In patients with MMA, the accumulation of propionyl coenzyme A could inhibit the activity of N-acetylglutamate synthetase and then decrease the synthesis of N-acetylglutamate, which is a natural activator of carbamyl phosphate synthetase.[12] Therefore, it is not surprising that plasma ammonia levels in the affected group were significantly higher than in the unaffected group (Figure 1). All of the affected patients were found to have a plasma ammonia level >200 μg/dL, and all the unaffected patients were found to have a level <200 μg/dL. There was no overlap between the affected and unaffected groups; therefore, plasma ammonia level was a good parameter to differentiate the affected from the unaffected newborns.

Due to the accumulation of a lot of organic acids in patients with MMA, it seems to be reasonable that the affected group had lower blood gas pH and bicarbonate levels than the unaffected group. However, because there was still some overlap between the 2 groups for these parameters, we do not think that these parameters are good enough for initial differentiation in MMA screening.

For comparison of liver function, aspartate aminotransferase (AST) levels of the affected group were significant higher than those of the unaffected group, but ALT levels were not (Figures 2 and 3). It is well known that different types of liver injury can increase the levels of ALT and AST to different ratios. For example, in viral hepatitis, the AST/ALT ratio is

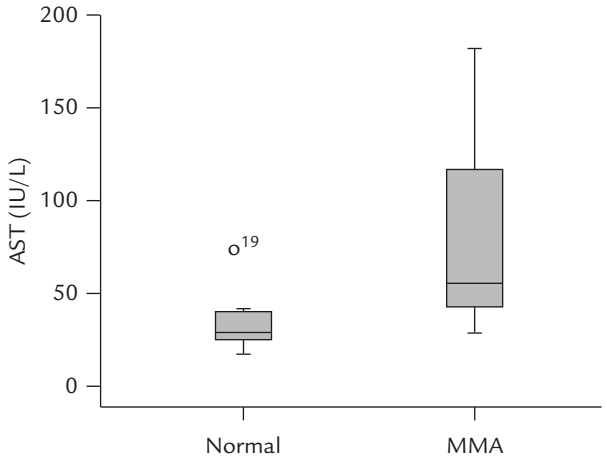

Figure 2. Comparison of aspartate aminotransferase (AST) concentrations between unaffected and affected groups.

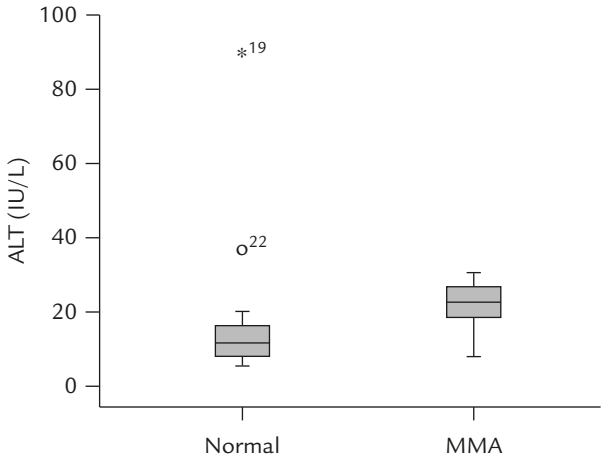

Figure 3. Comparison of alanine aminotransferase (ALT) concentrations between unaffected and affected groups.

usually < 1, but in alcoholic hepatitis, the ratio is usually > 1. This reminds us that some metabolites in MMA could perhaps cause injury to liver cells in a similar manner as alcohol or toxins.

There was no significant difference in gestational age between the affected and unaffected groups. However, the affected group had a significantly lower percentile of BBW than the unaffected group did. This suggests that there might be a certain level of prenatal effect of these metabolic inborn errors in fetuses in the affected group. However, in light of the small number of observations in this study, we cannot draw any conclusions, and more data are needed to prove the relationship between low BBW percentile and patients with MMA.

In conclusion, the referred newborns with elevated plasma C3/C2 ratios > 0.4 or ammonia levels > 200 μg/dL are highly suspected of having MMA. Emergency management should be established immediately to ensure a better prognosis.

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