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Case Report

Clinical Course of Two Newborns Affected by Cobalamin C Deficiency Diagnosed in the Pre and Post Newborn Screening Era

S ***Wong***, A ***Lin***, J ***Chow***, B ***But***, J ***Hui***

Abstract Cobalamin C deficiency is the best understood and most common phenotype within the group of intracellular cobalamin disorders. The clinical phenotype can range from a disastrous, heterogeneous multisystem disease presenting in the newborn period, to a milder disease with acute or slowly progressive neurological symptoms and behavioural disturbances. Here we report 2 cases of confirmed cobalamin C deficiency. The first case was diagnosed in the pre and the second case in the post newborn screening (NBS) era in Hong Kong. The dramatic difference in the clinical courses of these two patients highlight the importance of early diagnosis and treatment in improving the outcomes of affected individuals through a successfully implemented NBS programme.

Key words Cobalamin C; Homocysteine; Methylmalonic acidaemia; Newborn screening

Introduction

Cobalamin (or vitamin B12) is a cobalt-containing water soluble vitamin found in animal products such as milk and meat, and plays an essential part in amino acid metabolism.[1] This involves complex absorption, transport systems and multiple intracellular conversions for cobalamin to form two active co-enzymes, methylcobalamin in the cytosol and adenosylcobalamin in the mitochondrion.

Cobalamin C deficiency results from mutations in the MMACHC gene which lead to impaired conversion of dietary cobalamin to its two metabolically active forms, methylcobalamin and adenosylcobalamin. They are essential coenzymes to methionine synthase and methylmalonyl-CoA mutase, whose functional deficiency lead to homocysteinaemia combined with methylmalonic acidaemia. An increase of homocysteine with low levels of methionine in combination with methylmalonic acidaemia are biochemical hallmarks of cobalamin C deficiency.[2]

Without active metabolites of cobalamin, accumulation of methylmalonic acid and homocysteine leads to neurotoxicity, nephrotoxicity and vascular damage. Depending on the severity of the deficiency, the clinical phenotype can range from a disastrous, heterogeneous multisystem disease presenting in the newborn period, to a milder disease with acute or slowly progressive neurological symptoms and behavioural disturbances. Treatment is with parenteral hydroxycobalamin, betaine and folic acid. Haemolytic uraemic syndrome (HUS) as a consequence of vascular damage is one of the known neonatal presentations of cobalamin C deficiency.[3]

Dried blood spot tandem mass spectrometry-based

Department of Paediatric and Adolescent Medicine, Hong Kong Children's Hospital, 1 Shing Cheong Road, Kowloon Bay, Kowloon, Hong Kong SAR, China

S **Wong** (王雪娜) *MRCPCH, FHKAM (Paed)*
J **Hui** (許鍾妮) *FRACP, FRCP*

Department of Paediatrics, Prince Wales Hospital, The Chinese University of Hong Kong, 30-32 Ngan Shing Street, Shatin, N.T., Hong Kong SAR, China

A **Lin** (林安娜) *MRCPCH*

Department of Paediatrics, Queen Elizabeth Hospital, 30 Gascoigne Road, Kowloon, Hong Kong SAR, China

J **Chow** (周芷君) *MRCPCH, FHKAM (Paed)*
B **But** (畢慧文) *FHKAM(Paed), FRCPCH*

Correspondence to: Dr S **Wong**
Email: wongsn2@ha.org.hk

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newborn screening (NBS) enables babies with cobalamin C deficiency to be picked up with an elevated propionylcarnitine (C3) acylcarnitine. This finding together with a high C3/C2 carnitine ratio suggest a variety of disorders of propionate metabolism, including methylmalonic acidaemia, propionic acidaemia, and congenital cobalamin defects including cobalamin C deficiency.

The NBS programme for inherited metabolic diseases started in Hong Kong as a pilot programme since 2015. Here we report a case of cobalamin C deficiency who presented symptomatically with HUS diagnosed before the era of NBS in Hong Kong. The second case was diagnosed pre-symptomatically when the baby's NBS showed an abnormal result with an elevated C3 carnitine.

Case 1

A male neonate was born in 2014 by normal spontaneous vaginal delivery with a birth weight of 3.45 kg as the second child of unrelated Chinese parents with uneventful antenatal and postnatal courses.

He presented to the emergency department at 45 days of age with one-week history of progressive worsening irritability, shortness of breath, choking and coughing during feeds. Upon admission, examination revealed tachycardia, hypertension, marked pallor and generalised oedema.

A complete blood count revealed pancytopenia with Hb 4.6 g/dL, WBC $5.5x10^9$/L, platelets $76x10^9$/L. Serum chemistries showed metabolic acidosis with blood pH 7.25, pCO_2 5.0, BE -10.0 and renal failure with severe hyponatraemia (Na 115 mmol/L), hyperkalaemia (K 8.2 mmol/L), and mildly elevated urea of 8.9 mmol/L and creatinine of 42 µmol/L. Urinalysis was positive for protein and blood. His electrocardiogram was normal. Sodium bicarbonate, calcium gluconate and resonium were given for control of the hyperkalaemia and metabolic acidosis.

Multiple packed cell transfusions were given for correction of anaemia. Other pertinent initial investigations showed an increased reticulocyte count of 4.6%, elevated D dimers, low haptoglobin of <0.2 g/L and elevated C3 carnitine of 7.9 µmol/L (ref <0.88 µmol/L). Clotting profile was normal. Blood film revealed increased schistocytes with mild polychromasia and moderate thrombocytopenia. The clinical picture was consistent with microangiopathic haemolytic anaemia. He was transfusion dependent, requiring packed cells, platelet and fresh frozen plasma transfusions every 1-2 days.

Despite maximal supportive measures the patient's renal condition deteriorated with development of oedema, renal failure and persistent hypertension requiring multiple antihypertensives including IV hydralazine boluses and a labetalol infusion. Glomerular filtration rate was calculated to be only 13 ml/min/1.73 m^2 with persistent haematuria and proteinuria.

Because of the baby's unresponsiveness to supportive therapies, plasmapheresis was initiated. Unfortunately, it was complicated with bilateral acute subarachnoid haemorrhage and hydrocephalus requiring urgent decompression and craniectomy. He went on to developed seizures requiring midazolam infusion, and cardiopulmonary compromise with heart failure requiring inotropic and mechanical ventilatory support.

With the early age of atypical presentation of HUS, inherited causes were considered likely and metabolic investigations were initiated. Urine metabolic screen revealed a moderate increase in methylmalonic and methylcitric acids. Plasma homocysteine was markedly elevated to 107 µmol/L (ref 0-1 µmol/L). This finding together with the elevated cystathionine and low methionine of 3.4 µmol/L (ref 9-42) on the plasma amino acid profile suggested possible cobalamin C deficiency. The diagnosis was finally confirmed with targeted genetic testing of the MMACHC gene. Heterozygous MMACHC NM_015506.2:c.398_399delAA, p.(Gln133Argfs*5) and heterozygous MMACHC NM_015506.2:c.609G>A, p.(Trp203*) mutations were detected. Both mutations are predicted to result in truncated proteins and are known to be pathogenic.

Following commencement of subcutaneous hydroxycobalamin, our patient's blood counts stabilised, blood pressure normalised, renal function and urine output improved, seizures stopped and he was successfully extubated. His total duration of intensive care stay was 45 days. He has remained stable since discharge with no relapse and stable serum total homocysteine and methionine levels. Current medications include hydroxycobalamin, betaine, folinic acid and levocarnitine. He was diagnosed with global developmental delay and severe visual impairment on subsequent follow up. At his last follow up at 4.5 years of age, he continues to show developmental progress with a latest developmental assessment of motor development of around 24 months and language development of around 12-15 months.

Case 2

A male neonate was born at full-term by normal vaginal delivery with a birth weight of 2.92 kg. He was the second child of non-consanguineous Chinese parents with unremarkable family history. His antenatal history was unremarkable. He had perinatal pneumonia with respiratory distress soon after birth, requiring non-invasive ventilatory support for two days and a course of intravenous antibiotics.

The baby boy underwent newborn screening with dried blood spot collected at 24 hours of life. He was found to have elevated C3-carnitine 6.6 µmol/L (ref 0.51-2.5 µmol/L), elevated C3/C2-carnitine ratio 0.31 (ref 0.03-0.12), and low methionine level 4.68 µmol/L (ref 11-32 µmol/L). Clinically, the baby was feeding well. He did not have lethargy or seizure. He was well hydrated and tone was normal. Further workup for metabolic diseases was performed. Cobalamin C deficiency was diagnosed based on abnormal biochemical findings including raised C3-carnitine level 10.5 µmol/L (ref <0.88 µmol/L), elevated total plasma homocysteine level 149.5 µmol/L (ref 2.9-10 µmol/L) and low methionine level <3 µmol/L (ref 13-44 µmol/L). Both vitamin B12 level of 868 pmol/L (ref 145-569 pmol/L) and folate level of >45.4 nmol/L (ref 12.0- 30.0 nmol/L) were higher than the normal reference ranges and maternal vitamin B12 level was also normal. Urine organic acids showed marked increase in methylmalonic acid and small amount of methylcitric acid. Complete cell count was normal except for borderline thrombocytopenia 129x109/L. He did not have any metabolic acidosis, and renal function was normal.

Targeted genetic analysis confirmed cobalamin C deficiency with homozygous MMACHC NM_015506.2: c.609G>A, p.(Trp203*) mutations.

The baby boy was started on intramuscular hydroxocobalamin 1 mg injections daily since day 11 of life and oral betaine since day 12 of life. Betaine was gradually titrated up from 100 mg/kg/day to 250 mg/kg/day as tolerated. His homocysteine levels dropped significantly and methionine levels normalised with treatment (Figure 1). He was continued on normal feeds with no protein restriction. Hydroxocobalamin 1 mg injections was reduced to three times per week since four weeks of life when his homocysteine levels remained below 30 µmol/L. Dried blood spot showed normal C3-carnitine 0.95 µmol/L (ref 0.25-1.32 µmol/L) and methionine 13 µmol/L (ref 8-26 µmol/L) on day 14 of life. He was progressing well at six months old on his last follow-up. His growth parameters including head circumference and weight were on track with head circumference at 25th-50th centile and weight at 75-90th centile. His development was age appropriate. He had normal neurological examination with no seizures.

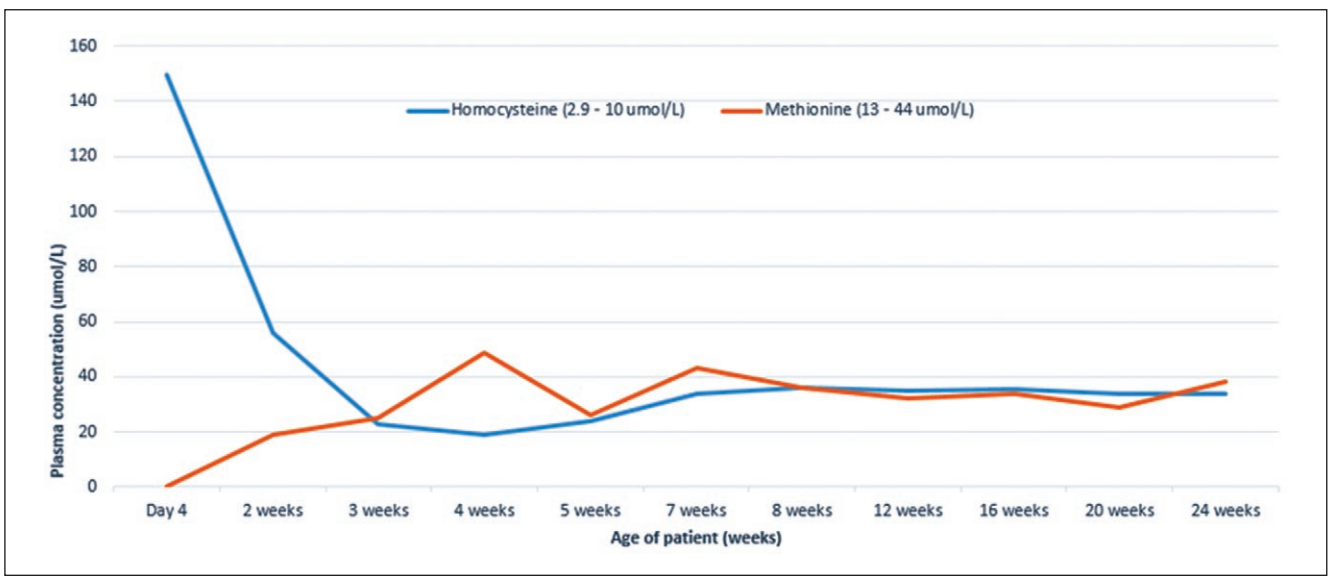

Figure 1 Homocysteine and methionine level.

Discussion

The incidence of cobalamin C deficiency estimated through newborn screening from the state of California in the United States of America is in the range of 1:67,000.[4] California is known to have an ethnically diverse population. When compared to ethnic Chinese population from Mainland China, the incidence of cobalamin C deficiency in China appears much higher with an estimated incidence of 1:16833.[5,6] It is the more prevalent type of methylmalonic acidaemia.[5] The availability of tandem mass spectrometry based newborn screening enables babies with cobalamin C deficiency to be picked up through elevated levels of propionylcarnitine (C3 carnitine) and low methionine. With the implementation of universal NBS for inherited metabolic diseases in Hong Kong, early diagnosis and early initiation of treatment can prevent irreversible neurological damage as well as many other disease related complications. This point was well demonstrated by the relatively uneventful clinical course of our second patient in contrast to the catastrophic clinical course of our first patient who was only diagnosed after symptomatic presentation.

Out of all the different types of cobalamin disorders, cobalamin C deficiency is the most prevalent among the Chinese population. The MMACHC gene mutation NM_015506.2:c.609G>A, p.(Trp203*) identified in both of our patients is the most frequent cobalamin C mutation among Chinese patients, affecting more than 50% of MMACHC alleles and may be associated with the early-onset phenotype.[7,8] With its high prevalence in the Chinese population, direct mutation analysis can be used for rapid confirmatory testing as well as potential development of targeted drug therapies in the future.

Though the overall incidence of cobalamin C deficiency is low, our 2 cases well demonstrated the benefit of NBS in improving the prognosis of these patients through early diagnosis and treatment. At the start of the pilot NBS programme in Hong Kong, only methylmalonic acidaemia due to methylmalonyl-CoA mutase deficiency was included among the 24 screened conditions. With the known higher incidence of cobalamin C deficiency among the Chinese population and the identification of local cases, cobalamin C deficiency will be officially added to the list of conditions screened in Hong Kong from October 2019 onwards.

Conflict of Interest

The authors have no conflicts of interest to disclose.

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