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BRIEF REPORT
Vitamin B_{12} Deficiency in Infancy: The Case for Screening

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The classic principles put forth by Wilson and Jungner are often applied to determine the suitability of a condition for universal newborn screening. The three cases described here portray the harmful effects of vitamin B_{12} deficiency in infancy. The challenges and opportunities of early recognition and treatment are highlighted. Screening newborns would allow early detection and prevention of severe neurological damage in vitamin B_{12}-deficient infants and enable diagnosis of unrecognized maternal pernicious anemia in asymptomatic mothers. However, lack of standardized methodology and screening cutoffs present challenges to the use of current tandem mass spectrometry technologies for screening. Pediatr Blood Cancer 2016;63:740–742. © 2016 Wiley Periodicals, Inc.

Key words: early detection; infants; neurological effects; newborn screening; vitamin B_{12} deficiency

INTRODUCTION

Vitamin B_{12} (cobalamin) has important physiologic roles in hematopoiesis, intermediary metabolism, growth, and early brain development.[1,2] Clinical features of B_{12} deficiency include macrocytic anemia lethargy, hypotonia, psychomotor delay, tremor, seizures, encephalopathy, irritability, weakness, diarrhea, stomatitis, glossitis, and failure to thrive.[1–4]

In children, B_{12} deficiency is most commonly caused by inadequate dietary intake or impaired absorption. Rare causes include the inborn errors of intracellular cobalamin transport or metabolism.[1,2] B_{12} deficiency due to decreased fetal stores and insufficient intake can occur in exclusively breastfed infants of mothers with undiagnosed B_{12} deficiency. Women with vegetarian or vegan dietary practices or unrecognized pernicious anemia are at risk.[2,5]

The precise incidence of neonatal B_{12} deficiency resulting from maternal deficiency is unknown.[2] In a retrospective American survey, Hinton et al. estimated it to occur in 0.88/100,000 births [95% confidence interval (CI) 0.60–1.26).[6] A higher incidence of 1:5,000 was detected by a newborn-screening pilot project in the Italian population.[7]

Because infants can present with severe neurological sequelae, the importance of early recognition has been emphasized in the literature.[3,4,8] However, B_{12} deficiency may be overlooked due to its nonspecific clinical features.[3,4]

CASE DESCRIPTIONS

Case 1 was an exclusively breastfed 6-month-old male who presented with profound hypotonia, hyperreflexia, and regression in psychomotor development. Magnetic resonance imaging of the brain and electroencephalography were normal. Laboratory studies revealed anemia, an elevated mean corpuscular volume (MCV) and low serum B_{12} level (Table I). His mother had low serum B_{12} (49 pmol/l; reference range 155–700 pmol/l), high plasma total homocysteine (90.1 μmol/l; reference range 4.1–9.9 μmol/l), high plasma methylmalonic acid (MMA) (21.2 μmol/l; reference range 0.05–0.27 μmol/l), and positive anti-intrinsic factor (anti-IF) antibody. The treatment resulted in rapid and sustained improvement in B_{12} levels. He regained the majority of his developmental milestones but remained mildly hypotonic at 10 months of age.

Case 2 was a 10-month-old breastfed female who presented with the parental concern of excessive somnolence. She had been meeting her developmental milestones at age-appropriate times. Complete physical and neurological examinations were unremarkable. An elevated MCV on complete blood count prompted the treating hematologist to order a serum B_{12} level that was very low (Table I). Her mother had low B_{12} (90 pmol/l), high plasma homocysteine (13.0 μmol/l), and positive anti-IF and antiparietal cell antibodies. The treatment was initiated, but she was lost to follow-up after the family relocated.

Case 3 was an exclusively breastfed 10-month-old male referred by his primary care physician for pallor and lethargy. His psychomotor development was delayed; he was unable to sit without support. Physical examination was unremarkable with the exception of axial hypotonia. Anemia and an elevated MCV prompted measurement of serum B_{12} level that was very low (Table I). His mother had low B_{12} (94 pmol/l), high plasma

Abbreviations: DBS, dried blood spot; IF, intrinsic factor; MCV, mean corpuscular volume; MMA, methylmalonic acid; NBS, newborn screening

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TABLE I. Laboratory Parameters of Index Cases at Presentation

Parameter (units) [reference range]	Case 1 (age 6 months)	Case 2 (age 10 months)	Case 3 (age 10 months)
Hb (g/l) [110–147]	100	72	112
MCV (fl) [71–90]	103	113	111
ANC (10^9/l) [0.6–5.1]	0.5	0.30	1.5
Platelets (10^9/l) [150–400]	152	339	547
Serum B_{12} (pmol/l) [155–700]	31	22	31
Plasma total homocysteine (μmol/l) [4.7–10.8]	156.5	130.7	72.5
Plasma MMA (μmol/l) [0.05–0.27]	19.5	15.6	37.8
C3/C2 on newborn DBS [<0.22]	0.26	0.14	0.22
C3 (μmol/l) on newborn DBS [<7.3]	6.20	3.63	5.21
C3–C16 on newborn DBS [No]	1.7	1.7	1.7

Hb, hemoglobin; MCV, mean corpuscular volume; ANC, absolute neutrophil count; MMA, methylmalonic acid; C3, propionylcarnitine; C2, acetylcarnitine; DBS, dried blood spot.

homocysteine (19.0 μmol/l), and positive antiparietal cell antibody. With the treatment, his psychomotor development had improved, but not normalized on follow-up at 16 months of age. His B_{12} levels remained normal after treatment completion due to an improved diet.

DISCUSSION

The three cases of infantile-nutritional B_{12} deficiency described herein presented clinically between 6 and 10 months of age, which is similar to other cases described in the literature.[3] All were breastfed by B_{12}-deficient mothers, and the two 10-month-olds had not been receiving age-appropriate complementary feeding. Although the Canadian Pediatric Society's current recommendations are to delay introduction of solid foods until 6 months of age, earlier introduction (around 4 months) of foods rich in B_{12} may be considered to prevent deficiency.[9,10]

Nonspecific clinical findings of B_{12} deficiency are often overlooked, leading to a delay in the diagnosis. Although the treatment reverses the hematological abnormalities quickly, the neurological manifestations may not completely resolve.[1,3,6,8,11,12] Two of the three described cases had mild to moderate psychomotor delay at presentation. In both cases, follow-up confirmed neurological improvement, but not normalization, after correction of the B_{12} deficiency.

B_{12}-deficient adults may be clinically and hematologically normal.[11,13,14] In all three described cases, the mothers were asymptomatic but were found to have low B_{12} levels and evidence of pernicious anemia on laboratory evaluation. Elevated MMA in blood and urine and, less commonly, total plasma homocysteine are sensitive, but nonspecific, markers of B_{12} deficiency.[7,14]

Population-based screening may provide an opportunity for early recognition of, and intervention for, infants with B_{12} deficiency.[7,11,15,16] Targeted screening of infants of vegan and vegetarian mothers is reasonable, but would only detect a minority of affected infants as many cases are due to impaired absorption of B_{12} from dietary sources, regardless of maternal nutritional practices. An approach of B_{12} screening in pregnancy by serum levels of B_{12} and/or MMA may be very cost effective, easy to implement, and helpful in preventing severe pregnancy-related complications in addition to benefits for the newborn.[3,17]

Universal newborn screening (NBS) programs are jurisdiction specific (typically overseen by state/provincial or national public health departments) and hold potential for maximal case ascertainment. NBS also offers the additional benefit of

TABLE II. Suitability of Population-Based Screening for Vitamin B_{12} Deficiency Based on Wilson and Jungner's Principles

Wilson and Jungner principle [20]	References supporting screening
1. The condition sought should be an important health problem.	[2–5,7–9]
2. There should be an accepted treatment for patients with recognized disease.	[2–5]
3. Facilities for diagnosis and treatment should be available.	[2–5]
4. There should be a recognizable latent or early symptomatic stage.	[2–5,7,8]
5. There should be a suitable test or examination.	[2–5]
6. The test should be acceptable to the population.	Not studied
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.	[2,7,8]
8. There should be an agreed-upon policy on whom to treat as patients.	[2–5]
9. The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.	Not investigated
10. Case finding should be a continuing process and not a "once and for all" project.	Not applicable

discovering asymptomatic, B_{12}-deficient mothers. Presymptomatic treatment could prevent the development of hematologic and neurologic dysfunction in these women.[14,17]

Considerable advances in NBS have occurred since the 1990s with the widespread adoption of tandem mass spectrometry.[18] With this technology, simultaneous quantification of multiple amino acid and acylcarnitine metabolites extracted from a dried blood spot (DBS) enables the diagnosis of certain inherited disorders of amino and fatty acid metabolism as a part of expanded NBS programs.

Propionylcarnitine (C3 acylcarnitine) accumulates in the setting of an inherited deficiency of the propionyl–CoA carboxylase or methylmalonyl–CoA mutase enzymes; both of which are targeted by NBS via C3 elevation. The mutase is adenosylcobalamin dependent; therefore, B_{12} deficiency results in a functional deficiency of this enzyme and C3 elevation. Campbell et al. have argued that the C3 level alone is not sensitive or specific enough to detect all newborns with B_{12} deficiency.[15] C3 levels may not be sufficiently high during the first few days of life when the DBS is collected.[15]

There is some evidence that the C3 to acetylcarnitine (C2) ratio may be better than C3 levels alone for detecting or identifying B_{12} deficiency.[19] Sarafoglou et al. were successful in detecting B_{12} deficiency in 11 cases by using lower cutoff levels for C3 (from >9.2 to >5.2 μmol/l), a C3–C2 (≥ 0.1) and C3 to palmitoylcarnitine (C16) ratios (≥ 2) followed by second-tier testing of MMA (≥ 5 μmol/l), and total homocysteine (≥ 15 μmol/l).[16] None went on to develop any symptoms or signs of B_{12} deficiency with cyanocobalamin treatment. Mothers of eight of the 11 newborns were diagnosed with B_{12} deficiency after their children were identified by NBS.

All three cases described in this report could have been identified if Sarafoglou et al.'s suggested cutoff for C3–C2 was used. The screening cutoffs suggested by Sarafoglou et al. are a good starting point, but refinement of the screening algorithm would require analysis of additional samples and careful consideration of the need for both maximal case ascertainment and minimization of false positives.

According to Wilson and Jungner's principles of screening, B_{12} deficiency may be a suitable candidate for inclusion in universal NBS panels (Table II).[20] Challenges, including the paucity of epidemiological studies defining the incidence of B_{12} deficiency in newborns, and lack of consensus on laboratory methods to maximize sensitivity and specificity exist.

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