European Journal of Clinical Nutrition (2017) **71**, 1013–1015

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CLINICAL CASE REPORT

An infant and mother with severe B_{12} deficiency: vitamin B_{12} status assessment should be determined in pregnant women with anaemia

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The vitamin B_{12} status of infants depends on maternal B_{12} status during pregnancy, and during lactation if breastfed. We present a 9-month-old girl who was admitted to the metabolic unit for assessment of developmental delay. She was exclusively breastfed and the introduction of solids at 5 months was unsuccessful. Investigations revealed pancytopenia, undetectable B_{12} and highly elevated methylmalonic acid and homocysteine. Methylmalonic acid and homocysteine normalised following B_{12} injections. Marked catch-up of developmental milestones was noted after treatment with B_{12}. Investigations of parents showed normal B_{12} in the father and combined B_{12} and iron deficiency in the mother. Maternal B_{12} deficiency, most likely masked by iron deficiency, led to severe B_{12} deficiency in the infant. Exclusive breastfeeding and a subsequent failure to wean exacerbated the infant's B_{12} deficiency leading to developmental delay. This case highlights the need for development of guidelines for better assessment of B_{12} status during pregnancy.

European Journal of Clinical Nutrition (2017) **71,** 1013–1015; doi:10.1038/ejcn.2017.85; published online 24 May 2017

INTRODUCTION

The vitamin B_{12} (B_{12}) status of infants depends largely on maternal B_{12} status during pregnancy, and during lactation if exclusively breastfed. Haemodilution, altered concentrations of B_{12} binding proteins, hormonal changes and active transportation of B_{12} across the placenta make the assessment of B_{12} status during pregnancy challenging. The evaluation of B_{12} status in infants is also challenging. Moreover, biochemical evidence of impaired B_{12} status has been reported in up to two-thirds of exclusively breastfed infants aged 6 weeks to 4 months.[1–3] We present the case of an infant with severe B_{12} deficiency, whose mother was also deficient.

CASE

A 9-month-old girl was admitted to the metabolic unit for assessment of developmental delay, abnormal movements (head dropping forward and arms moving up; 6–10 episodes per day) and pancytopenia with massive excretion of urinary methylmalonic acid (MMA).

She has been exclusively breastfed from birth. Parents attempted introducing solids at 5 months, but were unsuccessful. She previously smiled and was rolling front to back and back to front, however she had lost these skills. She was unable to sit unaided at the time of presentation. On admission the infant was anaemic, had brief myoclonic jerks but no epileptiform activity was seen on the electroencephalography and the electromyography was normal. Brain magnetic resonance imaging showed a generalised lack of white matter bulk with evidence of delayed myelination.

Blood investigations after admission confirmed pancytopenia, and revealed macrocytic anaemia, undetectable serum B_{12} and holotranscobalamin, low methionine, low iron, highly elevated serum MMA and total plasma homocysteine (Table 1). Urinary MMA was also elevated. Mild immunoglobulin M deficiency was present but no generalised hypogammaglobulinaemia or proteinuria.

A panel of genes related to disorders of B_{12} metabolism and transport was screened by next-generation sequencing. A heterozygous likely pathogenic mutation in the methylene tetrahydrofolate reductase (MTHFR) gene c.155G>A, p.(Arg52Gln) was identified (paternally inherited) but no second pathogenic variant detected. A heterozygous cubilin gene variant c.3604G>A, p.(Ala1202Thr), which is very poorly conserved across orthologous proteins was also detected (maternally inherited). However *in silico* analysis predicted this variant to be benign.

MMA and homocysteine normalised after 3 days of intramuscular hydroxocobalamin (B_{12}) 1 mg per day and 40 mg elemental iron daily. Parents reported a remarkable improvement in the infant's health. The infant was able to sit well unsupported, roll from both directions and had begun communicating her needs well. The infant continued to receive B_{12} injections and oral iron for 3 months.

FAMILY HISTORY

Investigations of the parents showed the father as B_{12} replete and the mother as B_{12} and iron deficient (Table 2). Her intrinsic factor antibody was negative, parietal cell antibodies positive.

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Received 23 January 2017; revised 20 April 2017; accepted 21 April 2017; published online 24 May 2017

Table 1. Selected results from the infant: samples taken at initial presentation and +3 days and +2 months post intramuscular hydroxocobalamin 1 mg per day, and 40 mg elemental iron daily

	Reference intervals/cutoffs	*Baseline baby*	*Post B_{12} injection (3 days later)*	*Follow-up (2 months later)*
WBC ($\times10^9$/l)	6–18	4.2	23.4	16
RBC ($\times10^{12}$/l)	3.60–5.20	1.91	2.03	5.22
HB (g/l)	105–135	68	72	128
PCV (l/l)	0.360–0.440	0.204	0.226	0.376
MCV (fl)	70–86	107	111	72
MCH (pg)	23.0–31.0	35.7	35.4	24.5
RDW %	11.0–16.0	24.0	27.5	15.2
PLT ($\times10^9$/l)	150–400	91	238	636
Neutrophils ($\times10^9$/l)	2.0–6.0	0.4	4.7	4.3
Lymphocytes ($\times10^9$/l)	5.5–8.5	3.7	8.9	9.6
Serum folate (nmol/l)	7.0–46.4	30.5		
Serum B_{12} (pmol/l)	138–652	<62		
HoloTC (pmol/l)	25–108	<5	>128	>128
MMA urine (μmol/mmol creatinine)	0–10	840		
MMA plasma (μmol/l)	<0.280	10	0.241	0.097
tHcy (μmol/l)	<15.0	133	11.3	5.1
Methionine (μmol/l)	10–53	5		
Ferritin (μg/l)	22–275		1233	
Iron (μmol/l)	14.0–25.0		9	12
TIBC (μmol/l)	41–77		62	51
Transferrin saturation (%)	18–71		15	24

Abbreviations: HB, haemoglobin; holoTC, holotranscobalamin; MCV, mean cell volume; MCH, mean corpuscular haemoglobin; MMA, methylmalonic acid; PCV, packed cell volume; PLT, platelets; RBC, red cell count; RDW, red cell distribution width; TIBC, total iron binding capacity; WBC, white blood cell count.

Table 2. Selected results of the infant's mother before and during pregnancy (performed at the local hospital) on presentation and post intramuscular hydroxocobalamin (initially 1 mg every alternate day for 2 weeks and then monthly), and oral iron (200 mg of ferrous sulphate per day)

	Reference intervals/ cutoffs	*8 months before pregnancy*	*At conception*	*At 2.5 months of pregnancy*	*At 7 months of pregnancy*	*9 months post pregnancy (at baby's presentation)*	*Follow-up (2 months post treatment)*	*Follow-up (half year later)*
WBC ($\times10^9$/l)	4.0–11.0	4.1	6.2	7.3	9.1	6.1		5.8
RBC ($\times10^{12}$/l)	3.95–5.15	*4.65	*5.24	*5.06	*4.28	5.02		5.08
HB (g/l)	120–150	**78	**141	**145	**130	121		139
PCV (l/l)	0.360–0.470	^0.260	^0.427	^0.433	^0.389	0.373		0.413
MCV (fl)	80–100	55.9	81.5	85.6	90.8	74		81
MCH (pg)	27.0–32.0	16.8	27.0	28.7	30.5	24.0		27.3
RDW%	11.0–16.0	^^19.1	^^15.6	^^16.0	^^14.8	15.3		14
PLT ($\times10^9$/l)	150–400	167	195	185	140	210		168
Neutrophils ($\times10^9$/l)	1.5–7.0	"2	"3.5			3.5		3.4
Lymphocytes ($\times10^9$/l)	1.2–3.5	†1.6	†1.8			2.0		1.9
Serum folate (nmol/l)	7.0–46.4	††21.7				23.1	21.7	31.2
Serum B_{12} (pmol/l)	138–652	‡98				83.5		1734
HoloTC (pmol/l)	25–108					<5	>128	335
MMA urine (μmol/ mmol creatinine)	0–10					<10		
MMA plasma (μmol/l)	<0.280					1.22	0.86	0.125
tHcy (μmol/l)	<15.0					30.2	6.5	
Ferritin (μg/l)	22–275	#2				8		19
Iron (μmol/l)	11.0–29.0					6.0	8.0	8.0
TIBC (μmol/l)	41–77					66	58	59
Transferrin saturation (%)	18–71					9	14	14

Abbreviations: HB, haemoglobin; holoTC, holotranscobalamin; MCV, mean cell volume; MCH, mean corpuscular haemoglobin; MMA, methylmalonic acid; PCV, packed cell volume; PLT, platelets; RBC, red cell count; RDW, red cell distribution width; TIBC, total iron binding capacity; WBC, white blood cell count. Local hospital reference intervals apply: *3.80–5.80, **120–160, ^0.350–0.450, ^^11.0–14.5, "2.0–7.5, †1.5–4.0, ††9.1–45.3, ‡ 96–590, #12–250.

The mother was 33 yrs and ostensibly fit, well and physically active. There is no history of neuropathy and the mother is not a vegan/vegetarian. However, she had a history of anaemia since childhood. She has two sons, aged 4 and 9 yrs. Her older son was diagnosed as anaemic age 7 yrs. Both the boys take multivitamins with iron. Both achieved normal developmental milestones. She also had three first trimester miscarriages prior to her pregnancy with this infant. She had been severely anaemic 8 months prior to

becoming pregnant with this infant (Table 2). At this time her B_{12} was 98 pmol/l and ferritin 2 μg/l. B_{12} status was considered normal at that time and her iron deficiency treated with ferrous fumarate, 210 mg three times a day. At conception, all full blood count (FBC) indices were normal except a slightly elevated red cell distribution width (RDW) 15.6% (Table 2). She was on intermittent ferrous fumarate supplementation with Pregnacare (a multivitamin containing 6 μg of B_{12} and 17 mg iron) during the first trimester. FBC markers, performed at ~2.5 and ~7 months of pregnancy were normal, except for RDW: 16% and 14.8%, respectively (Table 2). Serum B_{12} and ferritin were not measured during the pregnancy or post delivery.

Upon diagnosis of B_{12} deficiency, the mother received intramuscular hydroxocobalamin (B_{12}), 1 mg every alternate day for 2 weeks and oral iron. The mother continues to receive monthly B_{12} injections and 200 mg of ferrous sulphate daily, and her B_{12} and iron status are being regularly monitored.

DISCUSSION

Humans source their vitamin B_{12} from animal-derived foods, predominantly as hydroxocobalamin. The B_{12}-intrinsic factor complex is absorbed by the cubulin/amnionless receptors in the terminal ileum. The transcobalamin II carrier protein transports B_{12} to cells. Cells convert hydroxocobalamin into the two metabolically active forms: adenosylcobalamin and methylcobalamin. In the mitochondria, adenosylcobalamin is a co-factor for methylmalonyl-CoA mutase (mut), which converts methylmalonyl-CoA to succinyl-CoA. Genetic mutations affecting mut or adenosylcobalamin synthesis lead to high levels of MMA. In the cytosol, methylcobalamin and 5-methyltetrahydrofolate enable remethylation of homocysteine to methionine. Mutations affecting various stages in methylcobalamin synthesis (known as Cbl F, J, C, D, E and G) result in raised homocysteine and macrocytic anaemia.[4]

Mutations of the ileal receptors (Imerslund–Gräsbeck syndrome) or nutritional B_{12} deficiency reduce B_{12} overall, and manifest with high MMA as well as high homocysteine and macrocytic anaemia. In this infant, the combination of high MMA, high homocysteine, macrocytic anaemia and absence of pathogenic mutations of the ileal receptors made a nutritional B_{12} deficiency most likely. Vitamin B_{12} status of infants is largely dependent on the B_{12} status of mother. Studies have shown that breast milk alone is not sufficient to support the daily requirements of B_{12} intake, even from mothers with normal B_{12} status and especially if exclusive breastfeeding is continued beyond 6 months of age.[5,6] The investigations of the infant's mother revealed severe B_{12} deficiency. Her holotranscobalamin was undetectable. Other B_{12} markers were also consistent. Therefore, maternal deficiency was the most likely cause of B_{12} deficiency in the infant.

To the best of our knowledge, the mother received standard antenatal care as recommended by NICE guidelines.[7] In keeping with NICE guidelines, a screening for anaemia was performed using FBC at 2.5 and 7 months of pregnancy. The guidelines, however, do not advise investigations for B_{12} deficiency anaemia. They only state that if the haemoglobin level falls below 110 and 105 g/l (at 10 and 28 weeks, respectively), an iron supplementation is indicated. In keeping with these recommendations, B_{12} and iron status were not checked, despite abnormal RDW values reported as part of FBC screening. It is known that an elevated RDW is a marker for early iron, B_{12}, folate or combined nutrient deficiency anaemia.

Furthermore, 8 months prior to becoming pregnant, the mother's serum B_{12} was only 98 pmol/l. Of note, the cutoff of 96 pmol/l for B_{12} deficiency used by the laboratory is low in comparison with the most commonly used cutoff of 148 pmol/l.[8]

The assessment of B_{12} status during pregnancy continues to be challenging.[9] Pregnancy-related reference ranges are not used by most laboratories and no correction for hemodilution is made. In addition, the mothers of breastfed children who develop B_{12} deficiency are often asymptomatic.[10]

Maternal B_{12} deficiency, most likely masked by iron deficiency and a normal haemoglobin count on antenatal screening, led to severe B_{12} deficiency in the infant. Exclusive breastfeeding and a subsequent failure to wean exacerbated the B_{12} deficiency. This case highlights the need for development of guidelines for assessment of B_{12} status during pregnancy as well as public awareness of the risks of exclusive breastfeeding beyond 6 months of age.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We would like to thank the parents for their consent in submitting this report for publication. We also would like to thank the reviewers for their helpful comments.

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