ACTA PÆDIATRICA
PROMOTING CHILD HEALTH

Acta Pædiatrica ISSN 0803–5253

REGULAR ARTICLE

Clinical manifestations of infants with nutritional vitamin B_{12} deficiency due to maternal dietary deficiency

E Zengin, N Sarper (nazan_sarper@hotmail.com), S Çakı Kılıç

Department of Pediatrics, Division of Hematology, Kocaeli University, Kocaeli, Turkey

Keywords
Infants, Maternal dietary deficiency, Nutritional vitamin B_{12} deficiency

Correspondence
Nazan Sarper, M.D., Erenköy, Bilim sok. No9 Etik sitesi, A Blok. Daire:5, 34728, Kadıköy-İstanbul, Turkey.
Tel: +90-262-303-72-16 |
Fax: +90-262-303-80-03 |
Email: nazan_sarper@hotmail.com

Received
26 June 2008; revised 6 August 2008; accepted 5 September 2008.

DOI:10.1111/j.1651-2227.2008.01059.x

Abstract

Aim: In developing countries, nutritional vitamin B_{12} deficiency in infants due to maternal diet without adequate protein of animal origin has some characteristic clinical features. In this study, haematological, neurological and gastrointestinal characteristics of nutritional vitamin B_{12} deficiency are presented.

Methods: Hospital records of 27 infants diagnosed in a paediatric haematology unit between 2000 and 2008 were evaluated retrospectively.

Results: The median age at diagnosis was 10.5 months (3–24 months). All the infants were exclusively breast fed and they presented with severe nonspecific manifestations, such as weakness, failure to thrive, refusal to wean, vomiting, developmental delay, irritability and tremor in addition to megaloblastic anaemia. The diagnosis was confirmed by complete blood counts, blood and marrow smears and serum vitamin B_{12} and folic acid levels. The median haemoglobin level was 6.4 g/dL (3.1–10.6) and mean corpuscular volume (MCV) was 96.8 fL (73–112.3). Some patients also had thrombocytopaenia and neutropaenia. All the infants showed clinical and haematological improvement with vitamin B_{12} administration. Patients with severe anaemia causing heart failure received packed red blood cell transfusions as the initial therapy.

Conclusion: Paediatricians must consider nutritional vitamin B_{12} deficiency due to maternal dietary deficiency in the differential diagnosis of some gastrointestinal, haematological, developmental and neurological disorders of infants with poor socioeconomic status. Delay in diagnosis may cause irreversible neurological damage.

INTRODUCTION

The most common cause of vitamin B_{12} deficiency in infants is dietary deficiency in the mother (1). Due to restriction of vitamin B_{12} to foods of animal origin, maternal vegan diets cause severe deficiency in exclusively breastfed infants. Pregnant women who have been strict vegetarians for only a few years, and even omnivores who consume low amounts of animal products, are more likely to become vitamin B_{12} deficient during pregnancy and lactation and give birth to infants with diminished vitamin B_{12} stores. Exclusive breastfeeding of these infants further contributes to the deficiency. There are increasing number of reports concerning vitamin B_{12} deficiency in infants of vegetarians with the growing popularity of this diet and recent rise of exclusive breastfeeding practice in developed countries of Europe and the United States (2). The reason for maternal vitamin B_{12} deficiency in some developing countries is poor socioeconomical status, and infants presenting with severe haematological and neurological manifestations of vitamin B_{12} deficiency are more common than formerly appreciated (3,4).

Vitamin B_{12} is necessary for production of tetrahydrofolate, which is important for DNA synthesis. Vitamin B_{12} deficiency often presents with nonspecific manifestations, such as developmental delay, irritability, weakness and failure to thrive and is not easily diagnosed by paediatricians (5). Here, we report 27 infants with severe symptoms of nutritional B_{12} deficiency and try to emphasize its clinical and laboratory features.

METHODS

Twenty-seven infants with megaloblastic anaemia were diagnosed and followed up in a paediatric haematology unit of a university hospital between 2000 and 2008. The hospital records were evaluated retrospectively. The diagnosis was based on the nutritional history of the mother and infant, symptoms and physical findings of infants, haematological evaluation (macrocytic anaemia, bicytopaenia or pancytopaenia, macroovalocytosis, hypersegmentation of neutrophils in the peripheral blood smear and the megaloblastic changes in bone marrow precursors), decreased serum vitamin B_{12} and normal serum folate value. Complete blood counts and serum vitamin B_{12}, folate and ferritin levels of mothers were also evaluated. After the diagnosis, infants with severe anaemia leading to heart failure received packed red cell transfusion as the initial therapy. Then they were treated with intramuscular vitamin B_{12}, 150 μg every other day for 1 week, twice weekly for 2 weeks and once weekly for another 2 weeks. Vitamin B_{12} 1000 μg was also administered to mothers. Serum ferritin levels of the infants were high at presentation; however; oral iron supplementation 4–6 mg/kg/day was started in the second week and continued for about 3 months due to poor social and

economic status and iron-deficient diets of the families. Urine analysis of the patients was performed to detect any proteinuria attributable to Immerslund–Grasbeck syndrome. Data are presented as median (range) and percent.

RESULTS

The median age of 10 boys and 17 girls was 10.5 months (3–24 months). All the infants were born from mothers with inadequate animal-derived protein consumption and were exclusively breast fed. Mothers generally complained of their children's refusal of weaning. Only three of the mothers tried to feed infants animal proteins at the age of 7 months. The median age of mothers was 30 years (22–39 years), and only five mothers had some vitamin supplementation during pregnancy, but none of the mothers received vitamin supplementation during lactation. Twenty-five (92.6%) families had low socioeconomic status and were inhabitants of suburbs, and most of the fathers were workers in factories. The education level of the parents was generally poor, three mothers and one father were illiterate. The rest of the parents were graduates of elementary school except one father who was a college graduate. The mothers generally consumed very little amount of chicken monthly, egg, cheese or yogurt every other week and no milk and beef at all, although they were unaware of veganism. In addition, they had frequent pregnancies. Despite low income, 65.6% (15/27) of the families had more than two children.

The presenting symptoms and signs of the patients are shown in Table 1.

The laboratory characteristics of the infants and mothers are shown in Table 2. Ten infants had pancytopaenia, and 19 of the 27 patients (70.4%) had haemoglobin (Hb) level <7g/dL and 9 had severe anaemia with Hb <5g/dL. Peripheral blood smears revealed macroovalocytosis, anisocytosis, poikilocytosis and hypersegmentation of neutrophils. Two patients had dimorphic anaemia with relatively low mean corpuscular volume (MCV) (73 and 88 fL, respectively). Bone marrows of 15 patients were also evaluated and all showed megaloblastic changes. None of the infants had proteinuria. Although 11 mothers (57.9%) had low serum B_{12} levels, only one had pernicious anaemia. Two of the mothers also had iron deficiency anaemia. One of the infants and her mother were referred to our centre after administration of five doses of vitamin B_{12} and had reticulocytosis and high serum vitamin levels.

Table 1 Presenting symptoms and signs of the patients with maternal B_{12} deficiency

	Number of patients	%
Lemon-colour paleness	26	96.3
Refusal to wean	22	81.3
Apathy	18	66.7
Hypotonia	18	66.7
Failure to thrive	17	63
Motor retardation	16.	59.3
Papilla atrophy of tongue	16	59.3
No eye contact, social retardation	14	51.9
Vomiting	11	40.7
Irritability	8	29.6
Tremor	4	14.8
Athetoid head movement	1	3.6

Within 1 week of vitamin B_{12} administration, the infants showed signs of motor and mental improvement. They were more active and alert, began to roll over and developed eye contact. Weaning generally could be started at the end of the first week. The mothers were educated about nutrition, and printed materials were given. Thrombocytopaenia and neutropaenia values (except one patient) recovered at the end of the second week (median platelet count 461 000/mm^3 and granulocytes 3605/mm^3, respectively). At the end of the fourth week, 9 out of 15 patients (60%) with repeat blood count evaluations had an Hb value above 11 g/dL. Interestingly, three infants who were 15, 8, and 13month old at diagnosis with MCV values 96, 101 and 101 fL, respectively, had microcytic anaemia with MCV values 56, 68 and 69 fL, respectively. They had poor compliance with iron therapy and had normal Hb and MCV values after oral iron supplementation. During the 6- to 12-month follow-up, there was no relapse of the clinical and/or haematological features. All the infants began to walk and talk, and no developmental delay was observed during outpatient visits. There was no formal neurodevelopmental testing.

DISCUSSION

Pathogenesis of nutritional vitamin B_{12} deficiency in infants

Similar to our patients, exclusively breastfed infants of vegan and malnourished mothers, as well as infants of mothers with undiagnosed or untreated pernicious anaemia, are at an increased risk for megaloblastic anaemia (1,2). As Turkey is a country dependent on agriculture, plant-derived foods are cheaper than meat and dairy products. Mothers could describe the frequency of consuming animal-derived proteins, but could not give exact quantities about the composition of their diets. They usually consumed soups, cereal, vegetables and legumes and infrequently had very small amount of dairy products (yogurt and eggs). In the study of Speckeret al. (6), which showed low vitamin B_{12} concentrations in breast milk of vegetarian women, it was reported that these women were consuming 50–60% whole cereal grains, 5% soups, 20–25% vegetables and 5–10% beans and sea vegetables, no animal products, no dairy products or eggs. Similar to our mothers, generally, they usually did not have vitamin supplementation. In the present study, the mothers also neglected regular antenatal or well-baby visits due to poor education. In the recent years, similar cases have also been reported from our country (7,8).

In adults with low vitamin B_{12} intake, it may take 20–30 years for the clinical manifestations of the deficiency to appear. However, pregnant women who have consumed little amount of animal products in the preceding few years

Table 2 Laboratory characteristics of patients and their mothers

Investigation	Reference values for 3- to 24-month-old infants	Infants (n = 27), median range	Mothers (n = 15), median range
Haemoglobin (g/dL) (mean)	3–6 m 11.5	6.4	12.95
	7–24 m 12	3.1–10.6	7–14.7
Haematocrit (%) (mean)	3–6 m 35	17.5	ND
	7–24 m 36	9–32	
RDW (%) (range)	11.5–14.5	29	ND
		18–50	
Corrected reticulocytes (%)	3–6 m 0.7	3.07	ND
(mean)	7–24 m 1	0.2–11	
Platelet /mm^3	3–24 m	154 000	305 000
(range)	150 000–400 000	33,000–440,000	226 000–405 000
White blood cell /mm^3	3–24 m	6335	7372
(range)	6000–17 500	1500–20 800	5990–10 800
Granulocytes /mm^3	3–11 m 1000–8500	1400	4524
(range)	12–24 m 1500–8500	344–3500	3430–7890
MCV (fL)	3–6 m 91and 74	96.8	90
Mean and –2SD	7–24 m 78 and 70	73–112.3	(54.8–101)
Serum B_{12} (ng/mL)	3–24 m	100	155.5
(range)	140–700	41–328	100–1858
Folic acid (ng/mL)	3–24 m	15.1	9.63
(range)	3–35	5.16 – 44.4	4.65–28.8
LDH (U/L)	3–24 m	1683	ND
(range)	150–580	238–5982	
Ferritin (ng/mL)	3–24 m	165.5	ND
(range)	7–200	30–414	
Fe μg/dL (n = 11)	3–24 m	143	ND
(mean ± SD) (range)	68 ± 3.6 (16–120)	24–302	
Iron binding capacity (μg/dL)	3–24 m	253.5	ND
(n = 11) (mean ± SD)	300 ± 40	0–510	

ND = not defined.

Reference values are from Lanskowsky *Manual of pediatric hematology and oncology.* 4th ed. California: Elsevier Academic Press, 2005.

may give birth to infants who develop clinical and biochemical signs of B_{12} deficiency. Usual dietary requirements of vitamin B_{12} during pregnancy is about 2.6 μg/day, and requirements during lactation are slightly higher. Infant requirement is 0.4 μg/day, and the average breast milk vitamin B_{12} concentration is 0.42 μg/L (9). Specker et al. (6) reported that milk vitamin B_{12} concentrations were lower in women consuming a strict vegetarian diet compared to omnivorous mothers (231 ± 94 pM/L vs. 378 ± 75 pM/L). Milk vitamin B_{12} concentration was inversely related to the length of time a vegetarian diet was consumed. Milk vitamin B_{12} concentrations were correlated with maternal serum B_{12} concentrations and inversely correlated with maternal urinary methylmalonic acid (MMA) concentrations. Infant urinary MMA concentrations were inversely correlated with milk concentrations <362 pM/L. Deficient infants show full recovery when given even 0.1 μg oral vitamin B_{12} per day. An intake of less than 0.38 μg/day was reported to increase MMA excretion in the infant (3).

Clinical and laboratory features

Allen (3) reported that vitamin B_{12} deficiency due to maternal dietary deficiency generally manifests in breastfed infants at the age of 4–8 months, and these infants were born with inadequate B_{12} stores. During admission to our centre, 8 of 27 (29.6%) infants were 3- to 8 month old, and the rest were older (9- to 24 month old). However, this was due to a delay in the diagnosis because most of them displayed some symptoms in the previous months. As the symptoms are so nonspecific, the diagnosis is often missed by the paediatricians who are not familiar with the disease. Lemon-colour paleness due to intramedullary haemolysis and smooth-red tongue were frequent findings. This disease must be in the differential diagnosis of infants with failure to thrive and gastrointestinal symptoms. Refusal to wean and vomiting every food except breast milk were a common sign. None of our infants had a history of diarrhoea. One of the infants that was 10 month old at presentation had an earlier admission 2 months ago with vomiting. Although there was a mild megaloblastic anaemia (Hb = 10.3 g/dL, MCV = 99 fL, red cell distribution width (RDW) = 25.4), vitamin B_{12} deficiency was not considered. Surgery was consulted, and upper gastrointestinal contrast series was performed. He was discharged with diagnosis of gastrooesophageal reflux, and 2 months later, the patient presented in a very poor condition with severe anaemia (Hb = 3.9 g/dL, MCV = 90.3 fL, RDW = 36.7) and a weight loss of 2 kg. At that point, paediatric haematology was consulted. This infant was quite resistant to weaning and had to be fed via a nasogastric tube even during the second week of

treatment. Only then oral feeding could be started. The reference values of MCV for infants must be evaluated carefully. The maximum MCV values after 6 months may be calculated with the following formula MCV = 84 + 0.6 × age in years.

A rare but characteristic feature of B_{12} deficiency is tremor (7). Four of the patients (14.8%) had continuous tremor in the extremities and head. Another infant's admission was due to athetoid head movements, and the paediatricians' diagnosis was a seizure disorder. Tremor may also appear a few days after the initiation of cobalamin injection (10). In one patient, tremor increased after the second day of B_{12} injection, and with continued treatment, his tremor ceased. Head control, sitting, turning and walking were extremely delayed. In the literature, it has been reported that infants may have hypotonia, weakness or loss in deep tendon reflexes, no eye contact, apathy or irritability and severe encephalopathy (2,7).

Diagnostic tests, treatment and outcome

In countries with limited health-care resources, the efforts must be directed to prevention rather than treatment. If maternal diet and manifestation of the infant is suggestive for maternal nutritional B_{12} deficiency, therapy must be initiated after evaluation of blood count, blood and bone marrow smears and serum B_{12} and folic acid assays. Stabler and Allen (4) suggest that dramatic rise in haematocrit values and improvement of neurologic symptoms after B_{12} treatment are so specific that assessment of response can be the only diagnostic testing needed. We did not perform Schilling test and MMA and homocysteine measurements as they were not readily available. Lactate dehydrogenase (LDH) was also generally high. Despite vegan diets and low serum B_{12} levels (<200 pg/mL), the mothers usually did not have anaemia. If diagnosis depends only on abnormal Hb and MCV values, 30% of adults with B_{12} deficiency will be missed (5). The current methods of B_{12} analysis also measure to some extent inactive corrinoids in addition to vitamin B_{12} (4). Serum B_{12} levels of these mothers may be normal, but tissue levels or levels in their milk were probably deficient. MMA and homocysteine elevations in the plasma and urine are more sensitive parameters of preclinical vitamin B_{12} deficiency compared to serum cobalamin levels or haematological parameters (1). High amount of folate in the diet, iron deficiency and thalasemia trait may mask anaemia and macrocytosis (11). In this report, a few infants had normal MVC for age due to associated iron deficiency.

Vitamin B_{12} dosage in the treatment of infants with maternal or nutritional B_{12} deficiency is not well established. Several daily doses of 25–100 μg of vitamin B_{12} with potassium supplements are recommended as an initial therapy because hypokalaemia has been observed in adults with severe anaemia. A weekly dose of 100 μg is recommended for a month. Transfusion for partial correction of anaemia is also recommended for children with severe anaemia. Transfusion with limited volumes and diuretics plus oxygen prevents complications. Rapid administration of vitamin B_{12} with large doses before stabilization of severely anaemic infant may cause hypokalaemia due to immediate cessation of potassium loss from the cells (12,13). Oral iron was started at the end of the second week of B_{12} administration due to the expected increase in iron utilization with the beginning of effective haematopoiesis and iron-deficient diets of poor families. Parenteral administration of cobalamin was preferred due to the risk of vomiting the drug. With a similar treatment schedule, we observed no adverse effect and no need for potassium replacement.

Early neuromotor improvement is generally dramatic in maternal B_{12} deficiency of infants. There are some reports about MRI findings of these patients showing brain atrophy, reduction in myelin, moderate enlargement of ventricules and hypoplasia of corpus collosum, although long-term MRI follow-up examinations are generally lacking (1). Monagle and Tauro (14) reported severe long-term sequelae at 5 and 10 years in two patients with seizures at diagnosis. Early recognition of B_{12} deficiency is important to prevent permanent brain damage. Vitamin B_{12} should be added to iron and folate supplements given to pregnant and lactating women.

Paediatricians must be familiar with the clinical features of infants with maternal vitamin B_{12} deficiency in order to recognize these patients early. They must also evaluate MCV values more carefully because MCV values that are within normal values for adults and older children may indicate macrocytosis in infants. Patients presented here may be the visible part of the iceberg. Mild and moderate cases probably are not recognized. Motor improvement is generally rapid and dramatic, but some neurological damage may be irreversible, and children may not reach their full mental potentials.

As a conclusion, in infants with characteristic manifestations of vitamin B_{12} deficiency, if detailed nutritional history of infant and mother including vitamin supplementation is confirming animal-derived protein deficiency, treatment must be initiated at once following laboratory tests, which are available in routine laboratory. Complete blood count, reticulocyte count, peripheral blood smear, serum B_{12}, folic acid and LDH of the infant, in addition to urine analysis to detect any proteinuria attributable to Immerslund–Grasbeck syndrome (selective B_{12} malabsorbtion), must be performed. Bone marrow aspiration is optional as it is an invasive procedure. Complete blood count, serum B_{12} and folic acid must also be performed on the mother. A dramatic improvement of the symptoms and blood counts in the following 2 weeks will confirm the diagnosis.

References

1. Korenke GC, Hunnemal DH, Eber S, Hanefeld F. Severe encephalopathy with epilepsy in an infant caused by subclinical maternal pernicious anemia: case report and review of the literature. *Eur J Pediatr* 2004; 163: 196–201.
2. Lücke T, Korenke GC, Poggenburg I, Bentele KH, Das AM, Hartmann H. Maternal vitamin B12 deficiency: cause for neurological symptoms in infancy. *Z Geburtshilfe Neonatol* 2007; 211: 157–61.
3. Allen LH. Vitamin B12 metabolism and status during pregnancy, lactation and infancy. In:Allen LH, King J,

Lönnerdal B, editors. *Nutrient regulation during pregnancy, lactation and infant growth*. New York, NY: Plenum Press, 1994: 173–86.
4. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. *Annu Rev Nutr* 2004; 24: 299–326.
5. Rasmussen SA, Fernboff PM, Scanlon KS. Vitamin B12 deficiency in children and adolescents. *J Pediatr* 2001; 138: 10–7.
6. Specker BL, Black A, Lindsay A, Morrow F. Vitamin B-12: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. *Am J Clin Nutr* 1990; 52: 1073–6.
7. Katar S, Özbek MN, Yaramış A, Ecer S. Nutritional megaloblastic anemia in young Turkish children is associated with vitamin B12 deficiency and psychomotor retardation. *J Pediatr Hematol Oncol* 2006; 28: 559–62.
8. Çetinkaya F, Yildırmak Y, Kutluk G, Erdem E. Nutritional vitamin B12 deficieny in hospitalized young children. *Pediatr Hematol Oncol* 2007; 24: 15–21.
9. Allen LH. Impact of vitamin B12 deficiency during lactation on maternal and infant health. *Adv Exp Med Biol* 2002; 503: 57–67.
10. Ozer A, Turker M, Bakiler AR, Yaprak I, Ozturk C. Involuntary movements in infantile cobalamin deficiency appearing after treatment. *Pediatr Neurol*.2001; 25: 81–3.
11. Chan CWJ, Liu SYH, Kho CSB, Lau KHT, Liang YS, Chu WR, et al. Diagnostic clues to megaloblastic anaemia without macrocytosis. *Int J Lab Hematol* 2007; 29: 163–171.
12. Lanzkowsky P. *Manual of pediatric hematology and oncology*. 4th ed. San Diego, CA: Elsevier Academic Press; 2005: 47–50.
13. Whitehead VM, Rosenblatt DS, Cooper BA. Megaloblastic anemia. In:Nathan DG, Orkin SH, Ginsburg D, Look AT, editors. *Nathan and Oski's hematology of infancy and childhood*. 6th ed. Philadelphia, PA: WB Saunders Company; 2003: 419–55.
14. Monagle PT, Tauro GP. Infantile megaloblastosis secondary to maternal B12 deficiency. *Clin Lab Haematol* 1997; 19: 23–5.

